AIX-MARSEILLE UNIVERSITE

FACULTE DE MEDECINE DE MARSEILLE

ECOLE DOCTORALE DES SCIENCES DE LA VIE

UNIVERSITE CHEIKH ANTA DIOP

FACULTE DE MEDECINE

ECOLE DOCTORALE SCIENCE DE LA VIE,

DE LA SANTE ET DE L'ENVIRONNEMENT

**Le 02 Juillet 2020**

**THESE DE DOCTORAT EN COTUTELLE**

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**Sur le thème suivant :**

# Evaluation de la promotion de l'hygiène corporelle dans la prévention des maladies infectieuses en milieu rural au Sénégal

Membre du Jury de la Thèse :

| | |
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| Professeur **Jean Christophe LAGIER** | Président de Jury |
| Professeur **Marie KEMPF** | Rapporteur |
| Professeur **Max MAURIN** | Rapporteur |
| Professeur **Makhtar CAMARA** | Examinateur |
| Professeur **Mbacké SEMBENE** | Examinateur |
| Professeur **Didier RAOULT** | Directeur de thèse |
| Docteur **Cheikh SOKHNA** | Co-directeur de thèse |

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**Avant-propos**

Le format de présentation de cette thèse correspond à une recommandation de la spécialité Maladies Infectieuses et Microbiologie, à l'intérieur du Master des Sciences de la Vie et de la Santé, qui dépend de l'Ecole Doctorale des Sciences de la Vie de Marseille. Le candidat est amené à respecter des règles qui lui sont imposées et qui comportent un format de thèse utilisé dans le Nord de l'Europe et qui permet un meilleur rangement que les thèses traditionnelles. Par ailleurs, la partie introduction et bibliographie est remplacée par une revue envoyée dans un journal afin de permettre une évaluation extérieure de la qualité de la revue et de permettre à l'étudiant de commencer le plus tôt possible une bibliographie exhaustive sur le domaine de cette thèse. Par ailleurs, la thèse est présentée sur article publié, accepté ou soumis associé d'un bref commentaire donnant le sens général du travail. Cette forme de présentation a paru plus en adéquation avec les exigences de la compétition internationale et permet de se concentrer sur des travaux qui bénéficieront d'une diffusion internationale.

Professeur Didier RAOULT

Dédicaces

Je dédie ce travail

A Mon feu père qui nous a quitté en 2007. Les mots me manquent aujourd'hui pour vous exprimer ma profonde reconnaissance et témoigner votre amour envers vos enfants, ce présent travail m'est reconnu comme étant le fruit de vos efforts. Merci papa, que la terre vous soit légère.

A ma Mère, sans vous rien de tout cela n'aurait pu se faire. Que ce travail témoigne et exprime toute mon affection et ma reconnaissance pour les nombreux efforts et sacrifices que vous avez consentis pour moi.

A Ma petite famille, mon cher et tendre époux Mr BADJI et ma merveilleuse fille Banna pour votre amour inconditionnel, votre soutien. Merci de votre présence dans ma vie et d'être toujours là pour moi.

A mon oncle paternel et à mon homonyme qui m'ont élevé depuis toute petite et m'ont éduqué et inculqué certaines valeurs. Merci pour tous vos efforts consentis pour moi.

A toute la grande famille NDIAYE et ma belle-famille BADJI, pour votre amour et votre soutien inconditionnel durant toute ma formation.

A mes sœurs et frères vous êtes pour moi des exemples de persévérance, de courage et de

Générosité.

A mon amie, ma sœur Marièma SARR merci pour ton amitié et d'être toujours là pour moi.

A Tous mes amis pour votre amour et vos encouragements ;

Recevez à travers ce travail aussi modeste soit-il, l'expression de mes sentiments et de mon éternelle gratitude.

Remerciements

A mon Jury de thèse

Monsieur le Professeur **Jean Christophe LAGIER**

Je vous remercie infiniment de l'honneur que vous me faites en acceptant de présider le jury de cette thèse. Soyez assuré de mon profond respect et de ma sincère reconnaissance.

Monsieur le Professeur **Max MAURIN**, je vous remercie beaucoup d'avoir accepté de participer à ce jury. Soyez assuré de ma reconnaissance et de mon profond respect.

Madame le Professeur **Marie KEMPF**, je vous remercie d'avoir accepté de siéger au jury de cette thèse. Veuillez être assuré de ma sincère gratitude et de mon profond respect.

Monsieur le Professeur **Makhtar CAMARA**, je vous remercie beaucoup d'avoir accepté de participer à ce jury. Soyez assuré de ma reconnaissance et de mon profond respect.

Monsieur le Professeur **Mbacké SEMBENE** veuillez trouver ici mes sincères remerciements et ma reconnaissance pour avoir accepté de juger ce travail. Merci également de m'avoir initié à la recherche. Soyez assuré de mon profond respect et de ma sincère reconnaissance.

Monsieur le Professeur **Didier RAOULT, directeur de thèse**, je vous remercie infiniment pour m'avoir accueilli dans votre laboratoire et dirigé le travail de recherche. Votre rigueur, votre motivation m'ont guidé dans la réalisation de cette thèse. L'originalité et la richesse de vos approches m'ont beaucoup été d'une grande aide. Soyez assuré de mon profond respect et de ma gratitude.

Monsieur le Docteur **Cheikh SOKHNA, co-directeur de thèse** je vous remercie infiniment pour votre aide dans l'élaboration de ce projet, mais également pour votre soutien de tous les jours. Merci pour votre disponibilité. Vous avez fait preuve à mon égard de patience, d'exigence et de bienveillance. Soyez rassuré de toute ma gratitude et de mon plus sincère respect.

Docteur **Hubert BASSENE** de VITROME /SENEGAL pour son soutien infaillible, sa foi en moi, ses encouragements et conseils. Veuillez bien trouver ici mes sincères remerciements d'avoir supervisé ce travail depuis la collecte sur le terrain jusqu'à l'analyse des échantillons au laboratoire.

Aux personnes qui m'ont soutenu lors de ce travail

-Je vous remercie Professeur **Jean-Christophe LAGIER**, Docteur **Saber KHELAIFIA** de votre disponibilité, de votre soutien et de vos conseils tout au long de cette thèse.

-Je vous remercie Professeur **Mathieu MILLON**, de votre gentillesse et pour les formations "Atelier" écriture des articles de nouvelles espèces.

-Je remercie Docteur **MBOYING Fonkou Maxime Descartes**, pour l'analyse de mes données et pour les précieux conseils que vous m'avez prodigués. Merci pour votre disponibilité et toutes ces heures tardives et les week-ends que vous m'avez consacré. Soyez rassuré de toute ma gratitude.

-Ce travail a été rendu possible grâce à **l'équipe Culturomics** et **l'équipe de VITROME Dakar** que je remercie infiniment.

Merci chers collègues pour votre soutien, votre aide indispensable et précieuse tout au long de cette thèse.

**Résumé**

La peau humaine, une interface constante qui sépare l'intérieur du corps humain et l'environnement. Elle est le plus grand organe humain et constitue un environnement dynamique ; elle est constamment influencée par des facteurs internes et externes qui peuvent être à l'origine de la modification de sa communauté microbienne. Le microbiote de la peau humaine est complexe, et est constitué d'un mélange de différents groupes de micro-organismes ayant des types respiratoires différents. Malgré les progrès technologiques dans l'exploration du microbiote humain et les nombreuses études menées sur le microbiote du tube digestif, le microbiote cutané des sujets en bonne santé demeure un microbiote négligé. Jusqu'à récemment, la microbiologie de la peau était limitée à des études dépendantes de la culture, la plupart des échantillons provenant de pathologies telles que la sclérodermie, le psoriasis, l'eczéma et la rosacée. Malgré l'avènement de nouvelles techniques moléculaires qui a permis de mettre en évidence la diversité microbienne observée au niveau du revêtement cutané,le microbiote cutané reste toutefois compliqué à étudier car la peau présente des caractéristiques spécifiques en termes d'interactions écologiques avec l'environnement par rapport aux autres surfaces épithéliales. Dans cette thèse, nous avons utilisé l'approche culturomique pour explorer le microbiote cutané d'individus en bonne santé. Nous avons isolé 176 bactéries par culturomique. Parmi ceux-ci, nous avons ajouté 58 bactéries au répertoire du microbiote cutané, dont 6 nouvelles espèces isolées pour la première fois chez l'homme.

Nous avons ainsi augmenté le répertoire microbien associé à la peau humaine en découvrant et en décrivant de nouvelles espèces bactériennes qui sont décrites dans la dernière partie de cette thèse. Cependant la peau peut être une voie de transmission de bactéries pathogènes responsables de certaines maladies infectieuses qui sont l'une des principales causes de morbidité et de mortalité dans le monde. Toutefois, leur impact dépend largement de la situation géographique et du niveau de développement, y compris des conditions d'hygiène. Depuis

longtemps, l'hygiène des mains est reconnue comme l'un des outils les plus simples et les plus disponibles pour réduire le risque de transmission d'une infection et est considéré comme la principale mesure de prévention des infections. Dans cette partie nous avons également étudié le portage asymptomatique de certaines bactéries pathogènes sur la peau de sujets en bonne santé. Nous avons collecté 649 écouvillons auprès d'une population saine à Dielmo et Ndiop, dans les zones rurales du Sénégal. L'échantillonnage a été effectué sur la paume des mains. Une qPCR ciblant huit bactéries différentes a été effectuée sur 614 échantillons de peau après contrôle de l'extraction à l'actine. Nous avons détecté Streptococcus pneumoniae dans 33,06%, *Staphylococcus aureus* dans 18,08% et Streptococcus pyogenes dans 1,95% des échantillons. Un portage asymptomatique de *S. pneumoniae* a été détecté dans plus d'un tiers d'une population rurale en Afrique rurale, ce qui souligne la nécessité de développer des programmes de désinfection des mains afin de réduire la charge des infections. Dans cette partie de la thèse nous avons étudié l'impact de l'utilisation quotidienne du savon sur l'incidence des infections respiratoires, des fièvres et des bactéries pathogènes sur la peau.

Nous avons distribué du savon à la population du village de Ndiop (test) pour son hygiène quotidienne, mais pas à la population du village de Dielmo (contrôle). Nous avons enregistré quotidiennement les événements cliniques dans les deux villages et ont encouragé l'utilisation du savon dans le village test. 638 personnes ont participé à l'étude. Les taux d'incidence de la toux, de l'écoulement nasal et des fièvres ont diminué de manière significative en 2016 par rapport à 2015, contrairement à ceux de la diarrhée. En 2016, des réductions significatives des taux d'incidence de la toux, de l'écoulement nasal et de la fièvre ont été observées chez les enfants de moins de 15 ans dans le Ndiop. La prévalence de *Streptococcus pneumoniae*, *Staphylococcus aureus* et *Streptococcus pyogenes* dans la paume des mains a chuté de manière significative dans le village test. Nous avons pu démontrer que l'utilisation du savon à travers

le lavage des mains et une hygiène corporelle quotidienne réduit l'incidence des infections respiratoires, des fièvres et la prévalence des bactéries pathogènes sur la peau.

**Mots-clés** : Microbiote cutané humain, Culturomique, Nouvelles espèces bactériennes, Bactéries pathogènes, Portage asymptomatique, qPCR, Hygiène corporelle, Lavage des mains au savon, Infections respiratoires.

**Summary**

The human skin, a constant interface that separates the inside of the human body from the environment. It is the largest human organ and is a dynamic environment; it is constantly influenced by internal and external factors that can cause changes in its microbial community. The microbiota of human skin is complex, and is made up of a mixture of different groups of microorganisms with different respiratory types. Despite technological advances in the exploration of the human microbiota and the many studies conducted on the microbiota of the digestive tract, the skin microbiota of healthy subjects remains a neglected microbiota. Until recently, skin microbiology was limited to culture-dependent studies, with most samples coming from conditions such as scleroderma, psoriasis, eczema and rosacea. Despite the advent of new molecular techniques that have made it possible to highlight the microbial diversity observed in the skin lining, however, the skin microbiota remains complicated to study because the skin has specific characteristics in terms of ecological interactions with the environment compared to other epithelial surfaces. In this thesis, we used the culturomic approach to explore the cutaneous microbiota of healthy individuals. We isolated 176 bacteria per culturomic. Of these, we added 58 bacteria to the repertoire of the cutaneous microbiota, including 6 new species isolated for the first time in humans. We have thus increased the microbial repertoire associated with human skin by discovering and describing new bacterial species which are described in the last part of this thesis. However, the skin can be a transmission route for pathogenic bacteria responsible for certain infectious diseases that are one of the main causes of morbidity and mortality in the world. However, their impact depends largely on geographical location and level of development, including hygiene conditions.

and hygiene has long been recognized as one of the simplest and most available tools to reduce the risk of infection transmission and is considered the primary infection control measure. In this section we have also studied the asymptomatic carrying of certain pathogenic bacteria on

the skin of healthy subjects. We collected 649 swabs from a healthy population in Dielmo and Ndiop in rural Senegal. Sampling was carried out on the palms of the hands. A qPCR targeting eight different bacteria was performed on 614 skin samples after actin extraction control. We detected Streptococcus pneumoniae in 33.06%, Staphylococcus aureus in 18.08% and Streptococcus pyogenes in 1.95% of the samples. Asymptomatic S. pneumoniae carriage was detected in more than one third of a rural population in rural Africa, underscoring the need to develop hand disinfection programs to reduce the burden of infection. In this part of the thesis we studied the impact of daily soap use on the incidence of respiratory infections, fevers and pathogenic bacteria on the skin. We distributed soap to the population of the village of Ndiop (test) for their daily hygiene, but not to the population of the village of Dielmo (control). We recorded daily clinical events in both villages and encouraged the use of soap in the test village. 638 people participated in the study. The incidence rates of cough, runny nose and fevers decreased significantly in 2016 compared to 2015, in contrast to those of diarrhoea. In 2016, significant reductions in the incidence rates of cough, runny nose and fever were observed in children under 15 years of age in Ndiop. The prevalence of Streptococcus pneumoniae, Staphylococcus aureus and Streptococcus pyogenes in the palms of the hands dropped significantly in the test village. We were able to demonstrate that the use of soap through hand washing and daily personal hygiene reduced the incidence of respiratory infections, fevers and the prevalence of pathogenic bacteria on the skin.

**Keywords**: Human skin microbiota, Culturomics, New bacterial species, Pathogenic bacteria, Asymptomatic Carriage, qPCR, Body hygiene, Handwashing with soap, Respiratory infections.

**Introduction**

L'intérieur du corps humain et l'environnement sont séparés par une interface constante qui est la peau humaine (1). La peau est le plus grand organe humain et constitue un environnement dynamique ; elle est constamment influencée par des facteurs internes et exposée à des conditions externes. Ces facteurs intrinsèques et extrinsèques peuvent modifier la communauté microbienne de la peau (2). Jusqu'à récemment, la microbiologie de la peau était limitée à des études dépendantes de la culture, la plupart des échantillons provenant de pathologies telles que la sclérodermie, le psoriasis, l'eczéma et la rosacée (3)

Le microbiote de la peau humaine est unique et complexe, et est constitué d'un mélange de différents groupes de micro-organismes ayant des types respiratoires différents : des bactéries anaérobies facultatives, telles que *Propionibacterium acnes* ; des bactéries aérobies, telles que *Staphylococcus epidermidis* ; et des champignons, tels que *Malassezia furfur* (4). La composition du microbiote de la peau résulte d'un équilibre entre les conditions locales et les propriétés métaboliques de ses microorganismes (5). Résident ou transitoire, le microbiote de la peau varie quantitativement et qualitativement d'une personne à l'autre en fonction de l'âge, du sexe, du lieu, du système immunitaire et de certains facteurs physico-chimiques tels que l'humidité, le pH et la température (6).

La flore de transit est composée de champignons, de virus et de bactéries, dont la plupart sont des saprophytes. Ce sont des germes inoffensifs qui se nourrissent de la matière organique en décomposition de l'environnement (7). Cette flore peut également être constituée de bactéries pathogènes opportunistes qui peuvent provoquer des maladies si les défenses de l'hôte échouent. Ces germes peuvent contaminer temporairement la peau ou s'installer de manière plus permanente dans des endroits favorables en raison de l'humidité, des conditions de pH ou en cas de percée de la barrière épidermique . Cette flore ne s'installe pas en permanence à la surface de la peau, elle varie au cours de la journée et dépend des activités exercées et des variations des conditions environnantes. Toutefois, elle peut persister pendant des heures, voire des jours. Les espèces de transit les plus courantes sont Staphylococcus aureus, Pseudomonas aeruginosa et les espèces de Bacillus (7, 8).

La flore résidente est constituée de germes commensales, qui colonisent l'organisme sans provoquer de maladies. La composition et la distribution de cette flore sont relativement stables . Les espèces Gram+ à deux familles principales : les staphylocoques et les bactéries

corynéformes aérobies (Corynebacterium spp.) et anaérobies (Propionibacterium spp.) dominent cette flore résidente. Les staphylocoques à coagulase négative sont les espèces les plus courantes dans la flore cutanée normale, trois espèces prédominent : S. epidermidis, qui peut être isolé sur toute la peau, constitue plus de 90 % de la flore aérobie résidente présente sur la couche cornée. S. hominis qui est fréquemment isolé de la cavité axillaire, de la cavité inguinale et du périnée; S. haemolyticus qui se trouve principalement dans les bras, les jambes et les espaces interdigitaux. Les organismes corynébactériens comprennent les corynebactéries et les bactéries du genre Brevibacterium, les propionibactéries et les coquilles microscopiques. Les seules bactéries Gram négatives résidentes dans la peau sont du genre Acinetobacter (9).

Les changements socio-économiques et les différences de mode de vie qui en résultent ont un impact cruel sur notre microbiote. La plupart des études ont été menées dans les pays occidentaux (10). Seuls quelques rapports ont enquêté sur des populations vivant dans des zones climatiques différentes et dans des zones géographiques différentes (11-13).

Les maladies infectieuses sont l'une des principales causes de morbidité et de mortalité dans le monde. Toutefois, leur impact dépend largement de la situation géographique et du niveau de développement, y compris des conditions d'hygiène(14). L'hygiène des mains est depuis longtemps reconnue comme l'un des outils les plus simples et les plus effective disponibles pour réduire le risque de transmission d'une infection et est considéré comme la principale mesure de prévention des infections. Il est contrôlé dans de nombreux pays et se fonde sur les recommandations de l'Organisation mondiale de la santé (OMS) et les directives nationales (15). La propagation des bactéries transitoires par les mains joue un rôle important dans la transmission directe et indirecte de la maladie. Il est nécessaire de réduire le nombre de bactéries qui peuvent être transférées à des objets inanimés, des aliments ou d'autres personnes. Une seule source d'infection peut transmettre cette infection à d'autres personnes par contact direct ou indirect. Une étude portant sur la propagation de la salmonelle d'un membre de la famille malade à d'autres membres de la famille a révélé que le risque qu'un autre membre de la famille la contracte atteignait jusqu'à 60 % (16). Il est très probable que ces infections secondaires soient dues à une contamination croisée directe et indirecte dans la maison. Plusieurs études ont établi un lien entre les épidémies de shigellose et les pratiques de manipulation des aliments, en particulier le manque de lavage des mains (17). Un certain nombre d'études ont montré un portage et un potentiel de transfert manuel de certaines bactéries pathogènes présentes ou inoculées à partir de produits alimentaires contaminés (18).

Les agents pathogènes associés aux soins de santé peuvent être contractés par le biais de blessures infectées ou drainantes, mais aussi dans des zones fréquemment colonisées de la peau normale et intacte du patient (19, 20). Les zones périnéales ou inguinales sont souvent les plus colonisées, mais les aisselles, le tronc et les membres supérieurs (y compris les mains) sont aussi généralement colonisés (21, 22).

Les staphylocoques, entérocoques ou *Clostridium difficile* plus résistants à la dessiccation seraient probablement responsables de cette contamination. Une contamination de l'environnement inanimé a également été détectée sur les surfaces des stations de lavage des mains dans les salles et de nombreux organismes isolés étaient des staphylocoques (23). La capacité des microorganismes à survivre sur la paume des mains à différents moments a été démontrée par plusieurs études. Cette capacité à survivre pendant de longues périodes sur la paume des mains est un facteur de risque de transmission interhumaine de ces agents pathogènes. Par exemple, les mains d'un travailleur de la santé souffrant de dermatite psoriasique ont été colonisées par *Serratia marcescens* pendant plus de trois mois (24). Les études citées ci-dessous démontrent clairement que des mains contaminées pourraient être un moyen de propagation de certaines bactéries (25, 26). Une seule source d'infection peut transmettre l'infection à d'autres personnes par contact direct ou indirect. Le lavage des mains à l'eau et au savon est recommandé pour réduire le nombre de bactéries sur les mains (27) et il a été démontré qu'elle réduit le risque de maladie (28). L'hygiène des mains est la méthode d'intervention la plus importante pour réduire le risque de transmission croisée des infections (29). Le savon et l'activité mécanique du frottement des mains améliorent la capacité de l'eau à solubiliser et à éliminer la saleté et les agents pathogènes des mains (30, 31). Cette élimination physique des agents pathogènes réduit non seulement la transmission des agents pathogènes des mains d'une personne infectée à celles d'une personne non infectée, mais aussi la transmission des organismes infectieux des mains d'une personne non infectée aux voies respiratoires (32, 33). L'efficacité très similaire des savons antibactériens et ordinaires suggère que l'élimination physique des agents pathogènes des mains et de la peau avec de l'eau et du savon plutôt que l'activité antibactérienne du triclocarban est le facteur clé pour la prévention des infections respiratoires, de l'impétigo et de la diarrhée (32). Le lavage des mains, casserait la chaine de transmission des agents pathogènes respiratoires en milieu communautaire, permettant de réduire les taux de maladie respiratoire, mais aussi de réduire le nombre de personnes qui transmettent ces microorganisms et donc de limiter l'exposition des enfants vulnérables (32). Une étude au Pakistan a montré que le lavage des mains réduisait de 50%

l'incidence de la pneumonie chez les enfants de moins de 5 ans et de 53% l'incidence de la diarrhée chez les enfants de moins de 15 ans (32).

L'adoption de règles d'hygiène de base telles que le lavage des mains et du corps au savon a eu un impact sur l'apparition de fièvres et d'infections respiratoires (34-36). Elle a également eu un impact négatif sur le développement et la multiplication des bactéries pathogènes vivant sur la peau(37-39). L'étude du microbiote de la peau a commencé des années avant la biologie moléculaire. Les premières descriptions disponibles du microbiote cutané étaient donc basées sur des méthodes d'identification et de caractérisation des bactéries en fonction de la culture. Les communautés microbiennes décrites par les approches dépendantes de la culture sont incomplètes **(40).** De nouvelles approches techniques, outre la culture, sont évidemment nécessaires pour détecter les micro-organismes encore à cultiver, afin de nous permettre de dresser un tableau réel du microbiote de la peau (41). De plus, les methodes de culture ne permettent que de mettre en évidence les microorganismes vivants, tandis que les méthodes moléculaires détectent à la fois ceux qui sont viables et ceux qui sont non-viables. Ceci est d'une grande importance dans l'étude du microbiote cutané car la peau est en contact permanent avec les bactéries de l'environnement (42, 43).

Par ailleurs, Maldi-tof MS (matrix-assisted laser desorption and time-of-flight mass spectrometry) a progressivement évolué d'un outil analytique à une plateforme de diagnostic pour l'identification des pathogènes humains après une décennie de recherche intensive basée à la fois sur les protéines et les acides nucléiques (44). Bruker MALDI Biotyper (MALDI-TOF/MS) a également démontré son utilité pour l'identification des bactéries et des levures. Des rapports récents de laboratoires de diagnostic ont montré que, même parmi des groupes de micro-organismes très exigeants, environ 70 % des isolats cliniques peuvent être résolus avec confiance au niveau de l'espèce en utilisant le MALDI-TOF MS à haut débit.

La première partie de notre travail commencera par une revue de la littérature, établissant le répertoire des bactéries identifiées par culture et les méthods moléculaires de la peau humaine.

Dans la deuxième partie, nous montrons la place du concept microbial culturomique dans l'exploration du microbiote de la peau africaine des sujets sains . Son apport à la connaissance des bactéries connues de la peau et à travers le nombre des bactéries nouvelles ajoutées au répertoire des bactéries cutanées d'une part mais aussi des bactéries connues chez l'homme à travers la découverte de nouveaux taxa bactérien. La description de ces derniers a été réalisée

par l'approche de taxonogénomique qui combine à la fois des caractères phénotypiques et génotypiques mais également des données génomiques, indispensables dans l'ère de la microbiologie moderne. Ces descriptions se trouvent dans la cinquième partie de ce manuscript.

La troisième partie de ce travail, nous présentons le portage asymptomatique de *Streptococcus pneumoniae* détecté par qPCR sur la paume des mains de populations rurales au Sénégal. Nous montrons l'existence réelle de certaines bactéries pathogènes dont *S. pneumoniae* au niveau de la paume de la main.

Dans la quatrième partie de ce travail nous parlons de L'impact de l'utilisation quotidienne du savon dans les zones rurales du Sénégal sur les maladies infectieuses respiratoires, les fièvres et le microbiote de la peau. Nous évaluons l'impact de la promotion de l'hygiène corporelle dans la prévention des maladies infectieuses en milieu communautaire rural.

Nous résumons ce travail enfin avec une conclusion et perspectives.

# Partie I

## Elaboration du répertoire des bactéries de la peau humaine

Avant-Propos

Cette partie a été consacrée à l'établissement du répertoire des bactéries connues de la peau humaine. Un bref rappel sur l'anatomie de la peau ainsi que les types d'échantillons utilisés et enfin l'historique des méthodes de culture utilisée pour isoler les bactéries de ses sites ont d'abord été exposés. Ensuite les mêmes méthodes de recherche bibliographique utilisées par Hugon et al. en 2015 (45), révisées par Bilen et al. en 2018 (46) ont été appliquées pour établir le répertoire des procaryotes connus chez l'homme. Plus de 1000 espèces de bactéries différentes ont été isolées au moins une fois par culture ou détectés par les techniques de biologie moléculaire. Des informations relatives à la tolérance à l'oxygène, à la taxonomie, à l'origine géographique des échantillons et à la méthode d'identification utilisée ont été apportées pour chacune des bactéries listées de même que les pathologies associées à chacune de ses bactéries telles que décrites dans les publications scientifiques.Herein, we have listed a total of 1369 bacterial and archeal species cultured from the human Skin.

Article I: Revue

# **Repertoire of human skin microbiota:** A Systematic Review

Codou Ndiaye[1,2], Maxime Descartes Mbogning Fonkou[1], Jean Charles Dufour[1], Hubert Bassene[2], Marièma Sarr[1,2], Jean Christophe Lagier[1], Didier Raoult[1], Cheikh Sokhna[2,3]

**Repertoire of human skin microbiota: a systematic review**

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**Abstract**

The skin, a complex microbial ecosystem with interactions between microbial constituents, the microbes and the host. While the intestinal microbiota is currently in the spotlight, the skin microbiome is associated with several skin diseases. We conducted a systematic review to establish the currently known bacterial repertoire from the human skin microbiota using a semiautomated strategy. We included data from 71 countries and 793 articles. A total of 1562 bacterial and archeal species were identified mainly composed of *Proteobacteria* 628, *Firmicutes* 321, *Actinobacteria* 287, *Bacteroidetes* 111, *Spirochaetes* 36 and *Euryarchaeota* 30. *Streptococcus* 38 and *Corynebacterium* 35 were the most diversified reported genus. we compared the contribution of different methods of sample analysis and the list of bacteria obtained in patients with skin diseases and apparently healthy patients. In addition, we compared it with the other human repertoires. This repertoire could be valuable in elucidating the interactions between the skin microbiome and human health.

Keywords : Human skin microbiota, skin disease , heallthy skin, repertoire , Culture, Molecular methods

## Introduction

The skin, a complex microbial ecosystem with interactions between microbial constituents and between microbes and host (1).To protect the host from microbial infection, the skin harbors an integrated immune system with nonhematopoietically derived cells capable of secreting various substances, such as antimicrobial peptides, proteases, lysozymes, chemokines or cytokines that can induce the recruitment of immune cells (2).

The microbiota of human skin is unique and complex, and is made up of a mixture of different groups of micro-organisms: facultative anaerobic bacteria, such as *Propionibacterium acnes*; aerobic bacteria, such as *Staphylococcus epidermidis*; and fungi, such as *Malassezia furfur* (1). In 2018, Bilen et al. listed 2776 bacterial species that have been isolated from human beings by culture-based methods (3) . Over the last few years, advances in high-throughput sequencing have opened a window into the microbiome , and the renewal of bacterial culture methods has greatly contributed to the establishment of the prokaryotic repertoire associated with humans . As a matter of fact, it was estimated in 2018 that 2776 species were cultured from human beings, this spectacular increase being the result of the high through put culture techniques,in particular culturomics (3). Culturomics is the method allowing the description of the microbial composition by high-throughput culture . If the microbes from the gut remain the most studied human prokaryotes isolated by culture, intensive research is currently dedicated to the characterization of complex communities that inhabit environments such as the the skin and the lungs (4). Bacterial communities living on the skin surface appear to be diverse (5), but the total extent of bacterial diversity has not been adequately determined (6). Even if bacteria are common on all skin surfaces, we focused on the bacteria found on palm of hand because it is potentially one of the most dynamic microbial habitats of the skin due to its almost permanent exposure to environmental surfaces and the frequency of handwashing disruptions (7). The most popular methods are those based

on colony culture and counting methods (8). Culture and colony counting methods take a lot of time but give conclusive and unambiguous results (9). The study of the cutaneous microbiota began years before molecular biology. Therefore, the first available descriptions of the skin microbiota were based on culture-dependent methods for the identification and characterization of bacteria(10). The culture and seeding method is the oldest bacterial detection technique and remains the standard detection method. However, other techniques are needed because cultivation methods take far too long (10). Evans only identified colonies that represented more than 10% of all of the observed morphotypes (11). This type of approach surely failed to describe the actual structures of microbiota communities, as only a small fraction of bacterial species present in these communities can be cultured (5). Furthermore, preferential over growth of certain bacterial species is a known source of bias in quantitative descriptions of microbial communities (12). Thus, microbial communities described by culture-dependent approaches are incomplete (13)**.** In recent years, through initiatives such as the Human Microbiome Project, the scientific community has made great strides in cataloguing these microorganisms (14). One of the most common investigation sites is the skin, due to the ease of sampling and the potential role of the skin microbiome in the etiology of skin diseases, such as atopic eczema and psoriasis (15, 16). The study of the skin microbiome is challenging: healthy skin generally has a low microbial biomass, so several amplification steps are required before sufficient raw material is available for commonly used DNA sequencing approaches, increasing the risk of introducing sequence artifacts (e. g. chimeric DNA fragments) or detecting contamination of the environment or reagents used (17). The first studies used the amplification, subcloning and Sanger sequencing of the 16S rRNA gene, which is very well preserved and can provide sequence information over the entire length of the 16S rRNA gene in a single reaction. Although it is still the most complete method of bacterial identification, it is expensive and time-consuming. Next-generation

sequencing technologies (NGS) make it possible to identify members of the microbial community at a much lower cost and with a higher throughput (18).

Our objective was to establish a repertoire of the skin microbiota at the level of microbial species and their variations according to the detection method, geographical origin and skin-associated pathologies.

## Methods

### Search strategy and selection criteria

A systematic review following the PRISMA guidelines by using an automated search using the list of bacterial and archaeal species isolated at least once from humans, the List of prokaryotic names with Standing in the Nomenclature (http://www.bacterio.net) and the taxonomy of the National Centre for Biotechnology Information (NCBI) (http://www-ncbi-nlm-nih-gov.gate2.inist.fr/taxonomy/?term=) was conducted as previously reported (3, 19). The list thus established included 21,334 bacterial and archaeal species. These prokaryotes were searched in all studies indexed in PubMed from 1 January 1951 to 30 Avril 2019. The exact keywords and automatic search script are given in the additional data (Supplementary file 1). The query pattern was designed to express, with several combinations of keyword and text words, three notions that were expected to be present together in the retrieved articles: prokaryote denomination at the species level; skin or skin Diseases and studies carried out on human being. The eligibility criteria were as follows: a primary study with title and/or abstract reported in English (no language restriction on full text); concerns only human subjects; studies microbial diversity in skin regardless of the detection method used and reports primary results at the bacterial and archaeal species level. All studies that did not meet these inclusion criteria, systematic reviews, personal opinions and animal studies were excluded.

The primary objective was to determine the repertoire of bacteria and archaea detected in the human skin, regardless of the identification methods or the diseases status (without infection: healthy control, participant without skin infection – with infection, participant with skin diseases: infectious skin diseases, Skin ulcer, Fasciitis, Wound infection, Erythema, Exanthema, Foot diseases, Dermatitis, Leprosy, Panniculitis, Purpura, Pyoderma, Dermatoses, Skin Diseases, Vesiculobullous, Skin Diseases, Papulosquamous, Skin neoplasms and others diseases). We subsequently analyzed the variation of the repertoire according to different methods and infection status. The data collected were PMID, title, first author's name, year and country, analytical technique (culture or molecular or immunofluorescence technique or histological examination), diseases status (without infection: healthy control, participant without skin infection – with infection: infectious skin diseases, Skin ulcer, Fasciitis, Wound infection, Breast diseases, Erythema, Exanthema, Foot diseases, Dermatitis, Leprosy, Panniculitis, Purpura, Pyoderma, Dermatoses, Skin Diseases, Vesiculobullous, Skin Diseases, Papulosquamous, Skin neoplasms and others diseases and healthy participant without skin diseases). The detected bacterial or archaeal species were identified regardless of their abundance or frequency. The bacterial and archaeal species of the skin identified have been classified by taxonomy (phylum, class, order, family and genus) according to the list of prokaryotic names with standing in the nomenclature (http://www.bacterio.net) and the NCBI taxonomy website (http://www-ncbi-nlm-nih-gov.gate2.inist.fr/taxonomy/?term=).We recently observed that the gut dysbiosis is associated with an enrichment of microbes tolerant to oxygen and abnormal redox potential (20) Parte AC. LPSN – List of Prokaryotic names with Standing in Nomenclature (bacterio.net). In order to evaluate the proportion of microbial species tolerant to oxygen in the normal and abnormal (infected) human skin microbiota, we assigned each species its oxygen tolerance status: strict anaerobic or aerotolerant, according to

a previously published database (20). We subsequently compare the proportion of strict anaerobes in the skin repertoire in healthy controls and in skin diseases infection.

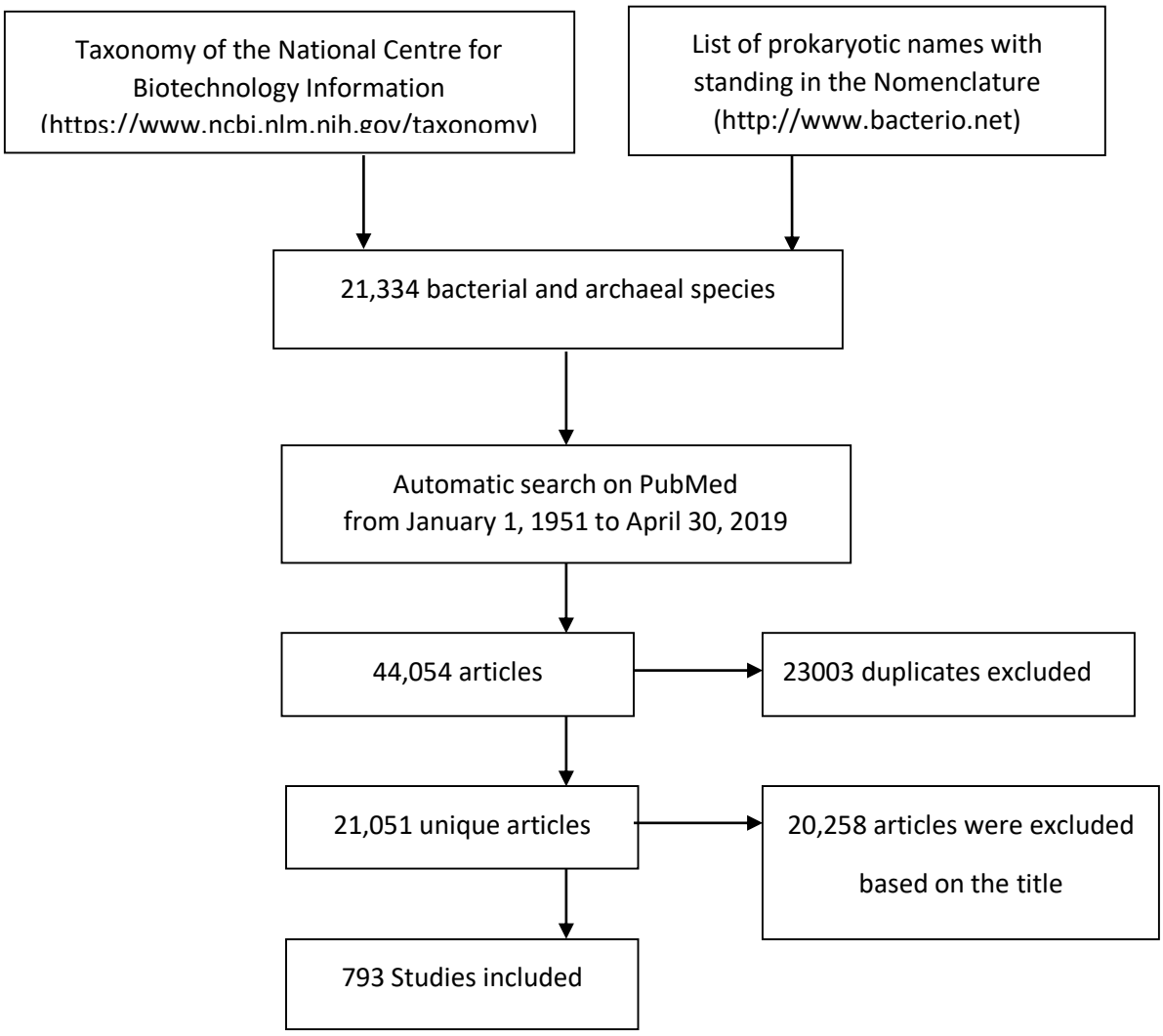


**Figure 1: study flowchart**

Description of the chronology of the search for publications to be included in this review. Using NCBI taxonomy and the list of prokaryotic names, 21,334 bacterial and archaeal species were included. The automatic search on pubmed between January 1951 and April 2019 resulted in the selection of 44,054 articles. Finally, 793 articles were selected after excluding duplicates and those whose titles did not match the criteria.

## Results

A total of 44,054 articles (Supplementary file 2) were identified corresponding after elimination of duplicates (an article can be automatically included many times if it mentions many species), to 21,051 unique articles recovered by automated search. A total of 20,258 articles were excluded based on the title or after reading the summary, so that 793 articles were identified by automated search (Figure 1). The 793 included articles were published between 1951 and 2019. More than 67.5% after the year 2000 and 42.7% in the last 10 years (Figure 2; Supplementary file 3).

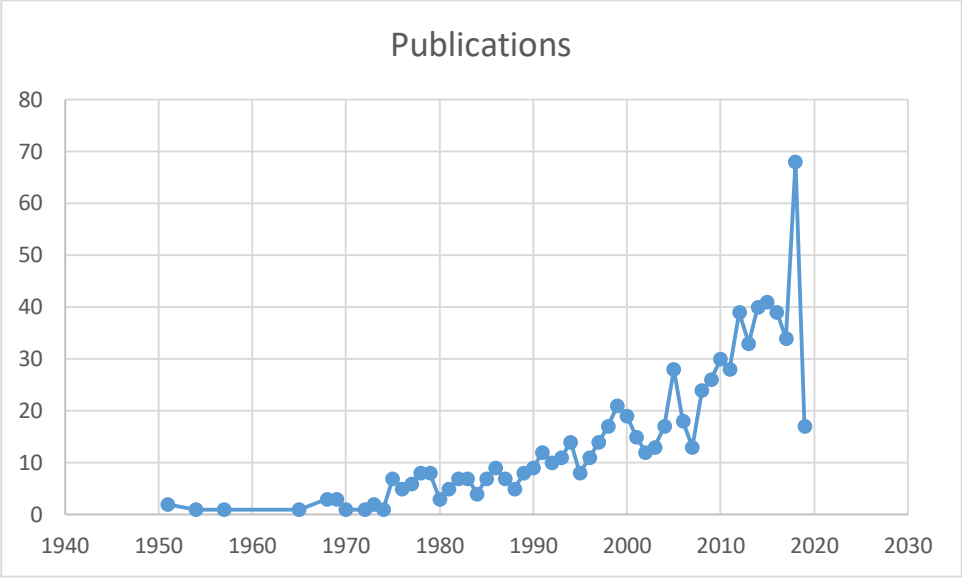


**Figure 2: number of skin microbiome articles published between 1951 to 2019.**

Summary of published articles on the cutaneous microbiota according to our search criteria. We note a significant increase in the number of publications from the year 2000 onwards.

The 793 included articles were published between 1951 and 2019. 71 countries contributed, and the most contributors were USA (199 articles), follow by France (71 articles), Japan 66, India (43 articles) and Spain (39 articles) (Figure 3 & Supplementary file 3). To date, microbiome studies have mostly focused on populations living in developed countries. Of the few studies that have included a more diverse array of populations, most have contrasted

urban populations in highly developed countries to populations in developing countries (21, 22),

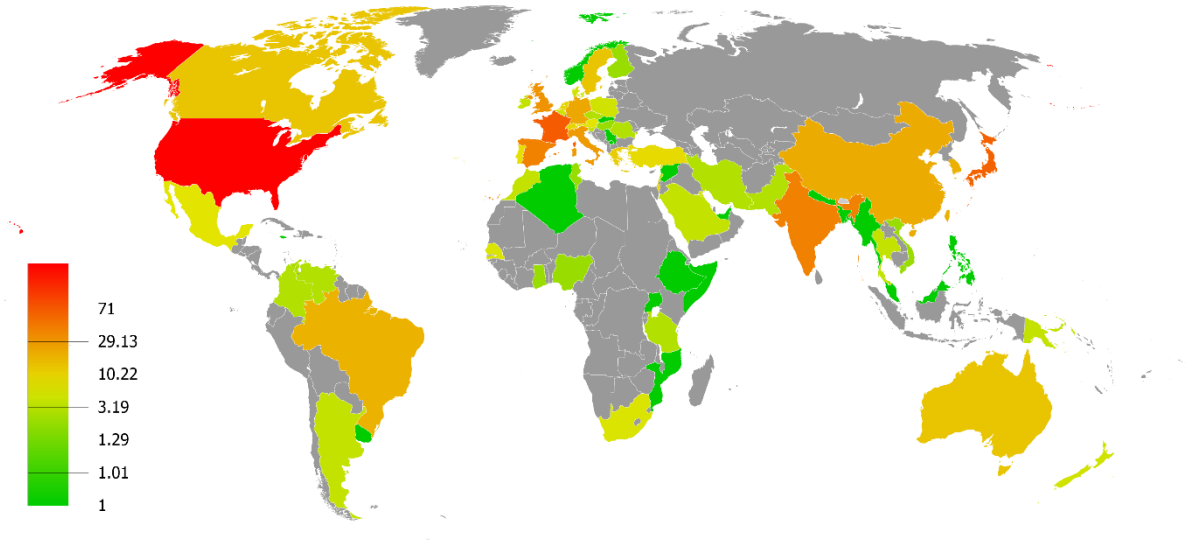


**Figure 3**: **Cartography of Articles published between 1951 to 2019 and proportion of contributors countries**

Several designs were identified including interventional and observational. Case reports account for 62% (492/793) of included studies.

Among 793 included articles, samples of many participants with various skin diseases were analyses. The top ten group of skin diseases was: infectious skin diseases with 350 studies, follow by Skin ulcer 76 studies, Fasciitis 58 studies, Wound infection 38 studies, Breast diseases 27 studies, Erythema 26 studies, Dermatitis 22 studies, Leprosy 12 studies, Skin neoplasms 10 studies. Only 77 articles study the samples of participants with no skin diseases (Supplementary file 5).

Regarding the technique used, culture was the most frequently used (558 studies) while 201 studies used molecular methods, with 32 using both culture and molecular methods. Other methods such as Histological examination 13, Microscopy 6, Immunofluorescence 4,

Serology 4, or Immunohistochemical 3 were also used to identify bacteria on the skin samples (Figure 4; Supplementary file 4). skin microbiology was limited to culture-dependent studies, with most samples from pathologies such as scleroderma, psoriasis, eczema, and rosacea (23)

**Complementarity of identification methods to studies skin microbiota**

The earliest descriptions of skin microbiota were based on culture-dependent studies. Skin was thought to harbor a limited number of microbial communities, primarily composed of aerobic cocci, aerobic diphtheroids, such as *Corynebacterium* and *Brevibacterium*, and anaerobic diphtheroids, consisting mostly of *Propionibacterium. acnes*, with minor representations of *Propionibacterium granulosum* and Malassezia yeast (24).

However, only in 2000, Rondon and collaborators coined the term “metagenomic libraries,” by generating libraries in Bacterial Artificial Chromosome (BAC) using DNA from soil samples. Furthermore, the authors also performed phylogenetic analysis of 16S rRNA sequences and identified several clones expressing heterologous genes in functional screenings using *Escherichia coli* as host. Since then, a large amount of data has been generated using metagenomic approaches and impacting different areas of high applicability in our society (25). Genomic studies have offered additional insight into the diversity of skin microbiota and allowed for quantitative analyses (26). Over the past 9 years, a high-throughput culture method known as culturomics has been developed and has significantly expanded the repertoire of cultured bacteria known to Human (27).

In this review, we found that, the culture methods allowed isolating 30.7% (420/1369) of the bacterial species detected in skin samples, while molecular methods detected 86.2% (1180/1369) (Figure 3; Table 1). Molecular methods seem more sensitive, but the two approaches are nevertheless complementary. Indeed, 69.2% (947/1369) species were detected only by molecular methods, while 13.5% (185/1369) were detected only by culture. The

overlap between the two methods was therefore very limited as only 14% (192/1369) of the species were detected by both techniques (Figure 4; Supplementary file 4). This significant discrepancy between culture and molecular methods has already been observed in all our studies where we compared microbial culturomics and metagenomics with a usual overlap of about only 15% (28-30).

All the archaeal species and candidate division TM7 was detected only by molecular methods, not by culture. Only 1/15 obligate intracellular bacterium *Lawsonella clevelandensis* was isolated and identified by culture, the rest was identified by molecular method.

*Rickettsia rickettsii* was detected in 5 different studies using histological examination or immunofluorescence or molecular methods but never in culture. *Treponema pallidum* was also detected in 7 studies by using immunohistochemical, microscopy or molecular methods but never isolated by culture. *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* was identified on skin samples only by molecular methods and serology in 6 and 2 studies, respectively. (Figure 4; Supplementary file 4). Overall, future studies will need to determine whether the many species detected only by molecular methods correspond to living microbes, in particular by using the microbial culturomics approach (28-30) and whether the few species detected only by culture correspond to species poorly detected by amplicon sequencing (relative abundance below the detection threshold of current machines[∼106 reads], primer bias, etc. (29) or whether they are contaminants.

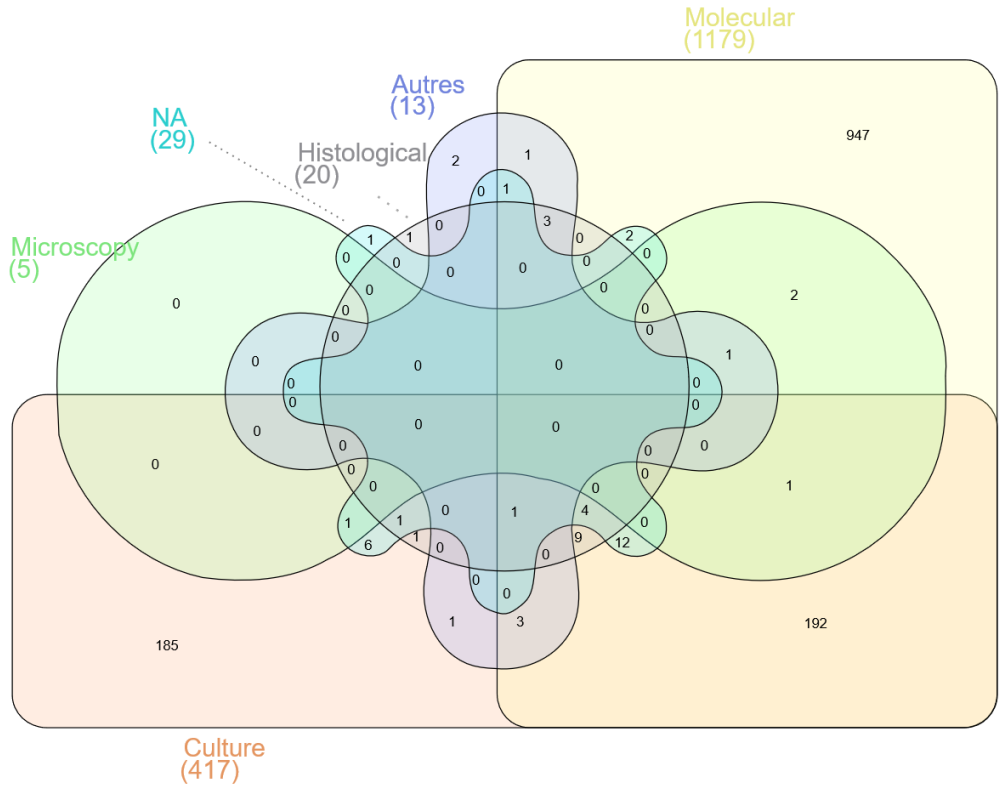


**Figure 4: Venn Diagram showed the shared number of species between different methods of identification**

**The human skin repertoire**

The 793 articles included yields 1327 bacterial and 42 archaeal identified at species level and 193 unclassified bacteria at species level. All were belonging to 31 phyla: *Proteobacteria* (628 species), *Firmicutes* (321 species), *Actinobacteria* (287 species), *Bacteroidetes* (111 species), *Spirochaetes* (36 species), *Euryarchaeota* (30 species), *Cyanobacteria* (21 species), *Tenericutes* (21 species), *Deinococcus-Thermus* (16 species), *Fusobacteria* (12 species), *Chloroflexi* (11 species), *Crenarchaeota* (10 species), *Acidobacteria* (8 species), *Planctomycetes* (8 species), *Verrucomicrobia* (8 species), *Chlamydiae* (6 species), *Chlorobi* (6 species), *Thermotogae* (6 species), *Aquificae* (3 species), *Nitrospirae* (2 species), Candidate division NC10 (1 specie), *Candidatus Saccharibacteria* (1 specie), *Chrysiogenetes* (1 specie),

*Deferribacteres* (1 specie), *Dictyoglomi* (1 specie), *Gemmatimonadetes* (1 specie), *Synergistetes* (1 specie), *Terrabacteria* group (1 specie), *Thaumarchaeota* (1 specie) and *Thermodesulfobacteria* (1 specie). There was member of 295 families and 666 genera identified (Table 1; Supplementary file 6). Genomic studies have offered additional insight into the diversity of skin microbiota and allowed for quantitative analyses. The same phyla found in human gut microbiota (*Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes*) have been found in skin, in addition to Cyanobacteria and other rare phyla (26). The ten most frequently reported bacterial species on skin were *Staphylococcus haemolyticus* (97 studies), *Staphylococcus aureus* (68 studies), *Escherichia coli* (48 studies), *Klebsiella pneumoniae* (37 studies), *Staphylococcus epidermidis* (37 studies), *Pseudomonasa eruginosa* (36 studies), *Proteus mirabilis* (33 studies), *Mycobacterium kansasii* (23 studies), *Finegoldia magna* (23 studies), *Mycobacterium marinum* (22 studies), *Serratia marcescens* (22 studies), *Enterobacter cloacae* (20 studies), *Enterococcus faecalis* (20 studies) *and Mycobacterium haemophilum* (20 studies) (Supplementary file 6). New identification methods have made it possible to discover the variability of the microbiota between the different anatomical zones of the skin: different skin areas harbour different bacterial communities. In the armpit, a pyrosequencing study found Staphylococcus to be the most represented genus (accounting for 35% of all reads (14), whereas a qPCR study identified *Corynebacterium* as the predominant genus (31).

The repertoire of skin was dominated by facultative anaerobic and aerobic bacteria. After exclusion of obligate intracellular, 20% (324/1562) of the 1562 species were strict anaerobes 20.7 % (282/1364) for species detected by molecular method, 17.2% (81/471) for species detected by culture (Table 1; Supplementary file 6). Overall, the proportion of strict anaerobes decreased with infection 19.6% (333/1692) compared with healthy participants 22.7% (272/1197), two-sided Chi square test, $p = 0.11$) (Supplementary file 5). The predominance of

bacteria tolerant to oxygen contrasts with the predominance of strict anaerobes in the gut microbiota. The anaerobic gut microbiota is characteristic of host maturation and health (32). The important members of this healthy human mature anaerobic gut microbiota correspond to *Akkermansiaceae* (*A. muciniphila*) and Clostridium cluster IV and XIV like *Ruminococcaceae* (*Faecalibacterium prausnitzii*) and *Lachnospiraceae* (*Mediterraneibacter gnavus* (formerly named *Ruminococcus gnavus*) (33), *Blautia obeum, Roseburia faecis*) (32). However, our results coincide with those of Togo and collaborators that found most of the bacterial species detected in breast and milk were facultative anaerobic or strictly aerobic bacteria, but the strict anaerobic bacteria usually associated with the gut were also detected (34).
The same phyla found in human gut microbiota (*Actinobacteria*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes*) have been found in skin, in addition to Cyanobacteria and other rare phyla (26).

Archaea are among the most sensitive to oxygen prokaryotes and are common inhabitants of the human gut, being key members of the host–microbial mutualism (20, 35). Caporaso et al. may have underestimated the importance of Archaea and particularly *Thaumarchaeota* on human skin (36). The study of Hannigan and colleagues in 2013 was the first to systematically show that Archaea, in particular *Thaumarchaeota*, are consistently present on human skin (37).


**Figure 5: Most represent genera from Human skin**

**Comparison between the skin microbial repertoires according to the pathologies**

### Healthy skin microbiota

From the studies focusing on participants without skin diseases, we repertories 1088 microbial species including 40 archaeal species and 1048 bacterial species. Bacteria that were strictly anaerobic were about 19.5% (213/1090). The 1088 species detected in healthy skin microbiota belonged to 31 phyla (Figure 6)., 279 families, the top ten was constituted by *Bacillaceae* 27, *Streptococcaceae* 27, *Burkholderiaceae* 26, *Staphylococcaceae* 25, *Enterobacteriaceae* 25, *Mycobacteriaceae* 25, *Lactobacillaceae* 24, *Flavobacteriaceae* 23, *Corynebacteriaceae* 23 and *Rhodobacteraceae* 19. And 571 genera, the top 10 are *Streptococcus* 25, *Corynebacterium* 23, *Staphylococcus* 23, *Lactobacillus* 22, *Bacillus* 18, *Burkholderia* 14, *Shewanella* 14, *Mycobacterium* 13, *Acinetobacter* 12, *Mycoplasma* 12. The details of the taxonomic classification are presented in the Supplementary file 5.

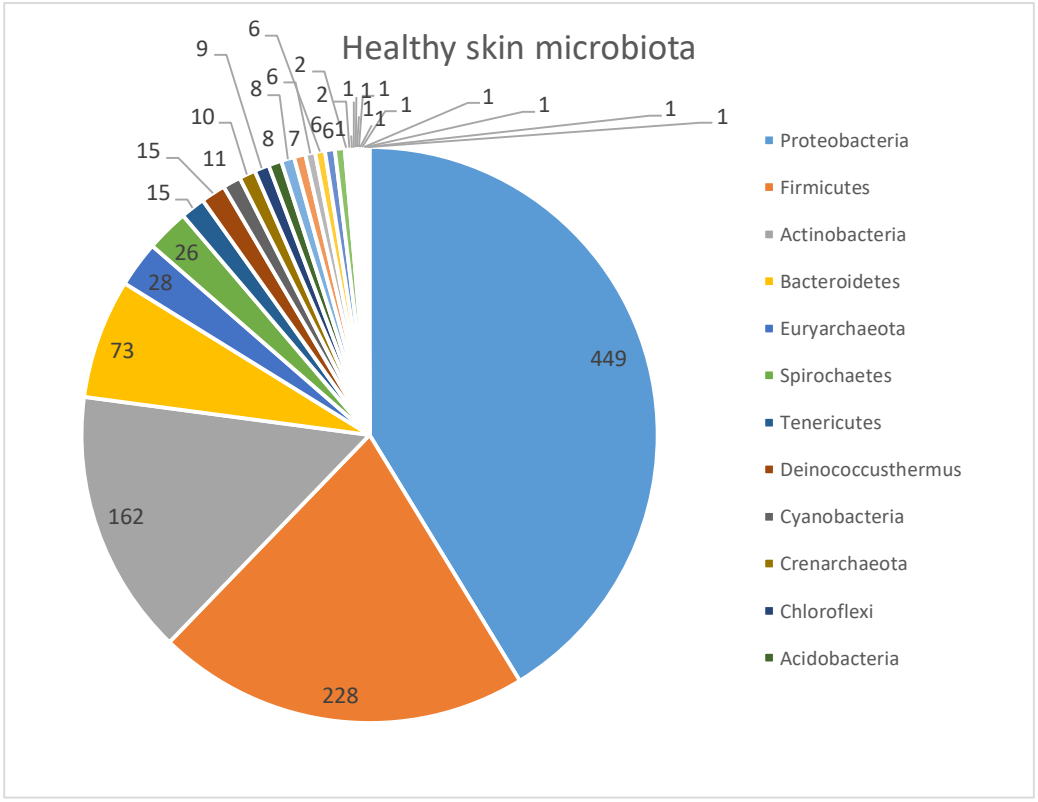

**Figure 6:** Bacterial species distribution according to their phylum in skin healthy microbiota. Based on the NCBI taxonomy and List of Prokaryotic names with Standing in Nomenclature classification reported in this repertoire

### Skin diseases microbiota

Of the 1369 bacterial and archaeal species, 481 were identified in skin diseases from participant samples (Supplementary file 5). The strict anaerobic were about 19.1% (92/481). The 481 species detected belonged to 9 phyla (Figure 7), 84 families (*Mycobacteriaceae* 56, *Enterobacteriaceae* 25, *Corynebacteriaceae* 24, *Staphylococcaceae* 24, *Streptococcaceae* 19, *Nocardiaceae* 18, *Neisseriaceae* 17, *Actinomycetaceae* 16, *Pasteurellaceae* 14 and *Prevotellaceae* 14) and 157 genera (*Mycobacterium* 29, *Corynebacterium* 24, *Staphylococcus* 24, *Streptococcus* 19, *Mycolicibacterium* 18, *Nocardia* 17, *Neisseria* 14, *Prevotella* 13, *Rickettsia* 12 and *Porphyromonas* 11). The details of the taxonomic classification are presented in the Supplementary file 6.

We noted that the healthy microbiota and skin diseases microbiota shared only 192 microbial species (Figure 8).

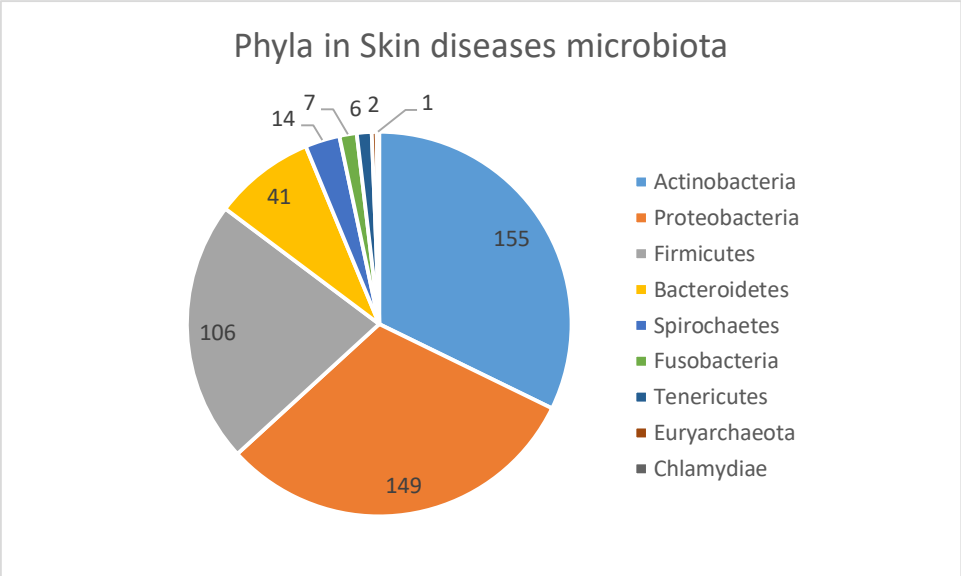


**Figure 7:** Bacterial species distribution according to their phylum in skin disease microbiota. Based on the National Center for Biotechnology Information taxonomy and List of Prokaryotic names with Standing in Nomenclature classification reported in this repertoire**.**

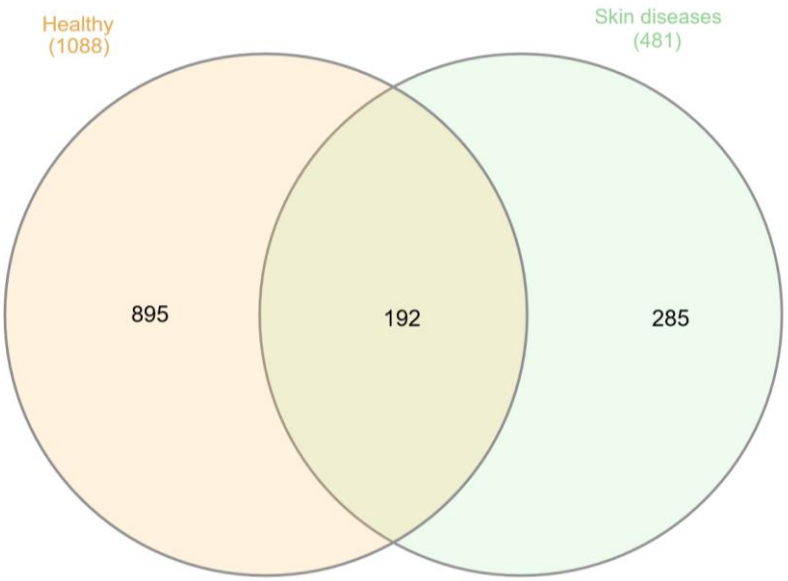


**Figure 8: Venn diagramm showing the number of microbial species shared between healthy skin microbiota and skin disease microbiota**

Before the advent of molecular techniques to characterize skin microbiota, the temporal and topographical diversity of the skin microbiota was still considered vast. Early studies produced variable results in bacterial quantity and taxonomy, hypothesized at the time to be a result of inherent topographical and temporal diversity of skin bacterial communities (38). In a comprehensive study, which cultured under both aerobic and anaerobic conditions, skin bacterial colonization differed between anatomical sites, and the highest bacterial loads was observed in sebaceous sites (38). Furthermore, skin colonization was dominated by a small group of taxa, including *Propionibacterium acnes* and *Staphylococcus epidermidis*. Years later, using a 16S rRNA sequencing approach in healthy adults, sebaceous regions were found to be preferentially colonized by Propionibacterium and Staphylococcus spp.; moist sites predominantly maintained Corynebacterium and Staphylococcus spp.; and dry sites, which, despite general variability and greater diversity, displayed a significant presence of β-*Proteobacteria* and *Flavobacteriales* (39). These authors identified 19 bacterial phyla, but skin was dominated by four phyla: the *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*. Interestingly, greater microbial diversity characterized skin microbiota, as compared with gut or oral microbiota of the same individuals. Key findings of these and other studies show that skin bacterial communities are generally diverse between individuals (5, 40) and may be influenced by ethnicity, lifestyle, and/or geography (41).

Although *S.aureus* likely in part contributes to disease pathogenesis, a role for the greater microbial community has recently been investigated. In a 16S-rRNA-based study that analyzed skin microbiota during the course of Atopic dematitis (AD) flares and improvement, a correlation between increased disease severity and decreased bacterial diversity was observed, along with altered microbial community structure in AD patients as compared with healthy controls (42). Bacterial community diversity was also shown to increase after standard AD treatment. Analysis of skin microbiota of a mouse model with a hypomorphic mutation

in St14, encoding the serine protease matriptase that regulates filaggrin processing, showed a selective shift in bacterial populations, with increased *Corynebacterium* and *Streptococcus* and decreased *Pseudomonas* species (43). These findings provide a link between filaggrin deficiency, a common genetic feature of AD, and changes in the skin microbiota. The mock community included a known skin commensal, *Staphyloccocus* species, which have been implicated in skin diseases such as atopic eczema where there is an increase in their abundance (44) and in psoriasis where reduced abundance has been reported (45).

The predominance of bacteria tolerant to oxygen contrasts with the predominance of strict anaerobes in the digestive microbiota. The anaerobic gut microbiota is characteristic of host maturation and health (32). The gut microbiota shape-up the skin flora as well. propionate, acetate, butyrate) are end products of dietary fiber fermentation in the gut and are known to take an important part in determining the microbial composition of the skin which is closely linked with the skin immune defense mechanisms (46-48). The *Cutibacterium* produces acetate and propionic acid in the gut, which are SCFAs. The propionic acid and its esterified derivatives suppress the growth of methicillin-resistant *Staphylococcus aureus* USA300 (in vit. In the meanwhile, skin commensals such as *S.epidermidis* and *C.acnes* that tolerate wider SCFA shifts than others.The findings of the study of Salem and colleagues suggest that there is a mutual interaction between the gut and skin (46).

**Comparison of the repertoire of skin with the other repertoires**

To identify the shared and specific microorganisms according to the anatomical niche, we compared the skin repertoire with the repertoires of the gut, oral cavity, vagina and breast and milk in humans. The human skin microbiota shared 27.97% (383/1369) of the species of its repertoire with the breast and milk, 30.24% (4141369) with the gut, 19.13% (262/1369) with the urinary tract, 19.36% (265/1369) with the respiratory tract, 14.17% (194/1369) with the oral cavity and 19.64% (269/1369)with vagina (Figure9; supplementary data 7).This is consistent with the fact that it has already been demonstrated that *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Staphylococcus* are shared between different anatomical niches of the human body such as the gut and the oral cavity (49, 50).

When comparing the skin microbial repertoire with other anatomical niches in the human body, we observed that four *Lactobacillus* were shared by all the anatomical niches studied (breast and milk, oral cavity, respiratory tract, gut, urinary tract and vagina), namely *L. casei*, *L. fermentum*, L. *gasseri* and L. *hominis* (Supplementary data 7). In adition *Bifidobacterium pseudocatenulatum* was detected in the skin, gut, vagina and breast and milk repertoires while no *Bifidobacterium pseudocatenulatum* was found in the oral cavity, urinary tract and respiratory tract repertoires.(Supplementary file 7)

Gram-positive *Bifidobacterium* and *Lactobacillus* are popular probiotic families. They lack lipopolysaccharides which cause inflammation and release active molecules that help maintain a healthy gut and skin. Probiotics modulate the immune system by encouraging regulatory T cell differentiation, and also by producing anti-inflammatory cytokines (TGF-β and IL-10) (51, 52). *Lactobacillus* has been shown to accelerate skin barrier recovery and inhibits skin inflammation related to substance P (53). *Bifidobacterium* exerts antipruritic effects by producing kynurenic acid, which is a metabolite that possesses neuroactive activity (i.e., antipruritic, antinociceptive) (54).

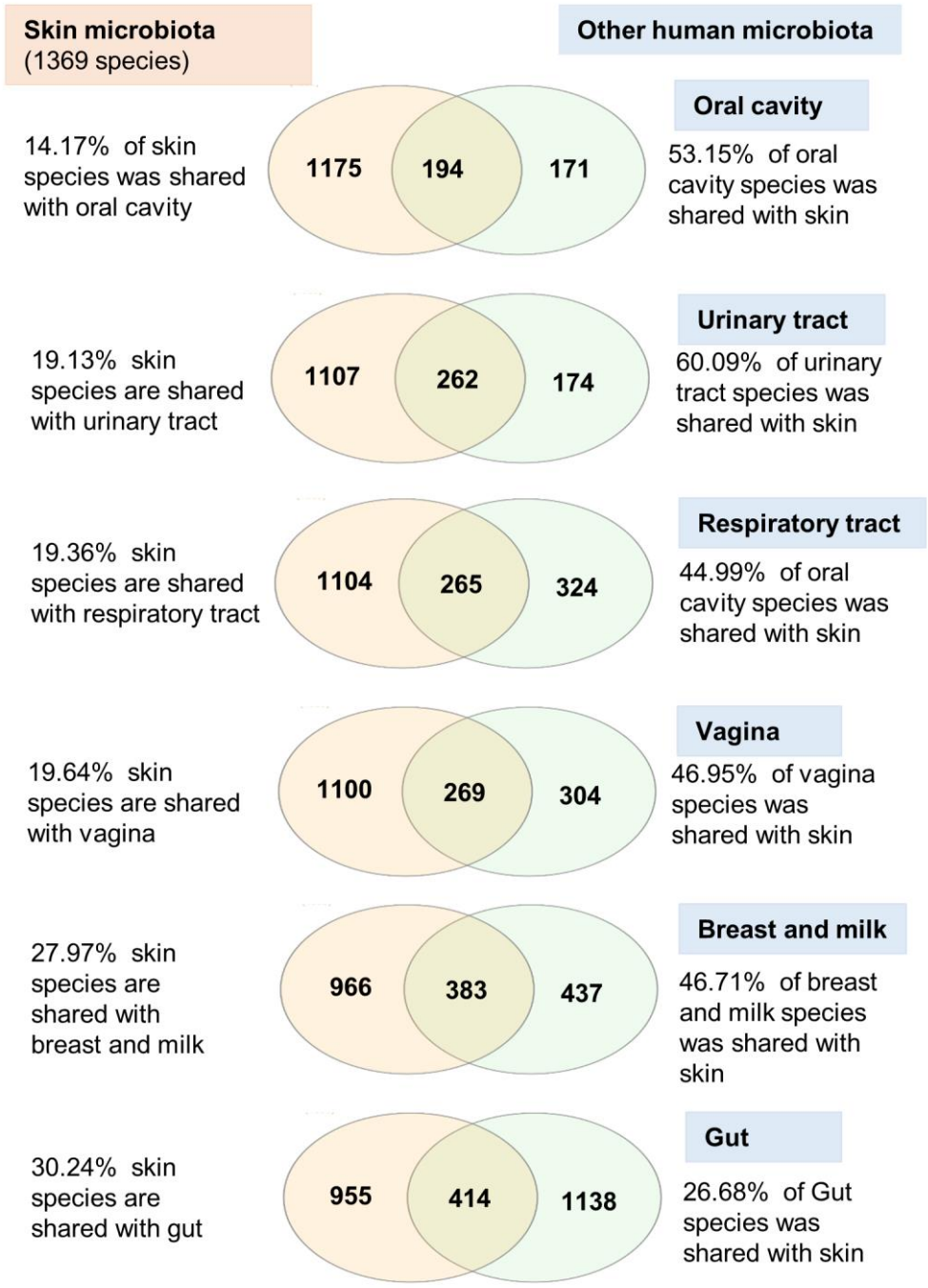
Skin microbiota
(1369 species)
Other human microbiota
14.17% of skin species was shared with oral cavity
1175
194
171
Oral cavity
53.15% of oral cavity species was shared with skin
19.13% skin species are shared with urinary tract
1107
262
174
Urinary tract
60.09% of urinary tract species was shared with skin
19.36% skin species are shared with respiratory tract
1104
265
324
Respiratory tract
44.99% of oral cavity species was shared with skin
19.64% skin species are shared with vagina
1100
269
304
Vagina
46.95% of vagina species was shared with skin
27.97% skin species are shared with breast and milk
966
383
437
Breast and milk
46.71% of breast and milk species was shared with skin
30.24% skin species are shared with gut
955
414
1138
Gut
26.68% of Gut species was shared with skin

**Figure 9.** Proportion of species from the human skin shared with other anatomical niches. Proportional Venn diagrams were performed using the online service https://www.meta-chart.com/

**Conclusion et Perspectives**

This review enabled to establish the repertoire of the human skin microbiota. We are nevertheless aware that such systematic search comprises several biases. Indeed, we found in this work a higher number of species cultured from human skin.

Our period of search is large and reflect evolution of microbiological techniques. In addition, certain taxa of bacteria would not have been isolated in earlier studies as it is only with molecular methods and better culture techniques that these have been identified. Another limitation of this review is that it does not consider the difference in quality between the included studies. It is possible that some members of the repertoire reported here are in fact contaminants. Moreover, many reports do not identify storage conditions and storage media. We did not consider collection techniques, cleaning of the skin. As we were primarily focused on the repertoire, we did not include any factors influencing the skin microbiota such as age, sex and ethnicity. In addition the number of observed species per sample or of intra- or intersample diversity indices and also we did not report the frequency of bacteria by individuals within included studies as we only considered the frequency among study groups. While individual meta-analysis is the gold standard, it is practically impossible because individual data are rarely provided in published studies. Future reviews on the skin microbiota should attempt to go beyond all these limitations. In fact, automatic search helped but did not replace humans to perform systematic reviews. Future culturomics studies could extend the repertoire of the Skin microbiota and thus change the paradigm: the human microbiome could be a connection of all human microbial niches such as the oral cavity, gut, urinary tract, vagina, respiratory tract and breastTherapeutic intervention for skin dysbiosis is in its infancy. Very few studies have evaluated the possibility of restoring dysbiosis and treating skin diseases by transferring microbiota to skin lesions. Authors treated a cohort of 25 patients with atopic dermatitis with a placebo or cream containing the commensal bacterium

Vitreoscilla filiformis for 30 days (53). They observed a significant decrease in the SCORAD score and pruritus in patients receiving active treatment compared to placebo. This appears to be clear evidence that topical application of microbiota could be used to restore a healthy skin microbiome and treat skin diseases associated with dysbiosis. We should also not forget that a small part of the human microbiome is made up of fungi, viruses and parasites (for example, Demodex folliculorum on the face). Their study still faces technical challenges; however, in the future, research may also discover the function of these microbes for skin health and their possible role in skin diseases. Therefore, understanding the role of the skin microbiome in its entirety, and its potential benefits, is of major value in clinical decision-making.

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# Partie II

# Exploration du microbiote de la peau africaine par approche culturomics

Avant-Propos

Plusieurs techniques sont actuellement utilisées pour explorer les microbiotes (respiratoire, intestinal, digestif, etc.). En dépit du fait qu'elle permet d'isoler et d'étudier une grande partie des éléments qui composent le microbiote, la culture bactérienne présente un certain nombre de limites symbolisées le plus souvent par les incertitudes sur les exigences des éléments du microbe. Les moyens de culture traditionnels ne permettent pas une description exhaustive du microbiome et les fonctions et les phénotypes de certaines bactéries restent inconnus du fait des difficultés rencontrées lors de leurs cultures. De nouveaux outils d'analyse des microbiotes « normaux » et pathologiques permettent néanmoins une meilleure approche phénotypique. La « Microbial Culturomics » a été utilisée en parallèle dans cette étude pour augmenter le répertoire des bactéries connus de la peau. La « Microbial Culturomics » est un concept innovant, développé depuis 2009, et basé sur une variation des caractères physico-chimiques des conditions de culture afin d'isoler des microorganismes jusqu'à lors incultivés. Les populations de deux villages (Dielmo et Ndiop) situés en milieu rural dans la région de Fatick (centre du Sénégal) ont été incluses dans cette étude. Les écouvillons cutanés de 402 sujets sains ont été mis en culture en conditions d'aérobie stricte sur 3 milieux différents. Au total 176 bactéries différentes dont 7 nouvelles espèces sur un total de 13927 colonies isolées. Ce travail a permis d'augmenter de 11.45 % le répertoire des bactéries connues vivant sur la peau humaine. La méthode de culture, bien qu'elle soit de plus en plus négligée au profit des méthodes de séquençage à haut débit, a montré beaucoup d'avantages dans exploration de l'écosystème cutané de l'homme. L'approche culturomic de la caractérisation de la diversité microbienne a considérablement modifié notre vision du microbiote cutané, soulevant par la suite de nombreuses questions importantes sur la relation hôte-microorganisme et sa pertinence pour les maladies de la peau.

Article 2

# Culturomics applied to explore the african skin microbiota

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Publié dans American Journal of Tropical Medicine in Hygiene

# Culturomics applied to explore the african skin microbiota

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Abstract word count: 197

Author summary: 174

Text word count: 3,399

**Abstract**

The rebirth of microbial culture was first promoted by environmental microbiologists through methods that reproduce the natural environment of microorganisms to promote their growth. For about 9 years now, culturomics a high throughput culture method has been developed and has allowed and has allowed to widen in a considerable way the repertoire of cultured bacteria known to man. An exhaustive database including list of microbes isolated by culture from human skin by performing a review of the literature was recently established. The aim of the present study was to use the culturomics approach to explore the african skin microbiota.

We have selected three culture media in aerobic conditions allowing us to isolate 176 bacterial species. This work enabled to increase the skin repertoire of 11.45% by adding 60 bacteria of which 7 were new species. The culturomics approach to characterizing microbial diversity has significantly changed our view of the skin microbiota, subsequently raising many important questions about the host-microorganism relationship and its relevance to skin diseases. Like the digestive microbiota, the composition of the skin microbiota can be related to the environment. This study demonstrates the need to continue to explore the skin microbiome using culture approaches.

**Key words:** Skin microbiota; African subjects; Environment; Aerobic conditions; Culture media

## Introduction

Human skin is the first layer of defense against infectious microorganisms and toxic agents. Skin is the largest human organ, and is a dynamic environment; constantly impacted by internal factors and exposed to external conditions. These intrinsic and extrinsic factors can alter the microbial community on the skin.[1] Until recently, skin microbiology was limited to culture-dependent studies, with most samples from pathologies such as scleroderma, psoriasis, eczema, and rosacea.[2] However, commensal bacteria are detected everywhere on humans, with up to $1\times10^7$ bacteria per $cm^2$ on the skin.[3] Although the culture-based approach is still common, many microorganisms are difficult to cultivate and are therefore under-represented or undetected in culture-based surveys. To date many studies of the human microbiome have focused on the gut and oral microbiomes. There are increasing numbers of skin microbiome studies, however, sampling has rarely focused on the hands.[4, 5]

Human hands are a conduit for exchanging microorganisms between the environment and the body. Hands can harbor pathogenic species, including meticillin-resistant *Staphylococcus aureus* or *Escherichia coli*; particularly within high risk environments, such as healthcare and food-handling settings. [6] Since hands are important for intrapersonal and interpersonal transfer of microorganisms, as well as environmental transfer, the dynamics of hand microbial communities and factors impacting them are of considerable importance.[7]

According to early descriptions of the skin microbiota based on culture-dependent studies, the skin contained a limited number of microbial communities, mainly composed of aerobic cocci, aerobic diphtheroids, such as *Corynebacterium* and *Brevibacterium*, and anaerobic diphtheroids, mainly composed of *P. acnes*, with small representations of *Propionibacterium granulosum and Malassezia.*[8] The skin has been included among the anatomical sites to be explored in the vast "Human Microbiome Project".[9]

Research on the skin microbiome lags far behind that on the intestine,[10, 11] where it is commonly accepted that it is unbalanced, with no causal link to many health problems.[12] The preservation and encouragement, not destruction, of the intestine microflora is now recognized as essential for overall health.[13-15] The crucial role that the skin plays in overall health has just been realized.[16] The discovery of this mechanism using biodiversity was inspired by the exceptionally high human intestinal microbial diversity found in the rural areas of Burkina Faso.[17] We found a total lack of conclusive evidence linking a particular type of dominant bacteria to skin health or disease, as humans have a wide intra- and interpersonal variation in the microbial composition of the skin[18-20] where each individual has a "virtually unique microbiota".[21-23]

While the origins of the human milk microbiome are not exactly known,[24] several hypotheses for its establishment have been proposed. Bacteria present in human milk may be derived from the surrounding breast skin flora,[25] or the infant's oral cavity microbiota.[26] Retrograde backflow during nursing or suckling may also lead to bacterial establishment in the mammary ducts.[27]

A study carried out in Africa shows a predominance of *Staphylococcus epidermidis* and *Streptococcus* spp. in breast milk, the main groups of bacteria isolated by culture. These results are similar to those of studies in European women in which Staphylococci, Enterococci and Lactococcocci were the most frequently isolated bacterial species in breast milk.[28-30]

These results suggest that environmental factors may not have a large influence on the bacterial composition of human milk.[31]

The rebirth of microbial culture was first promoted by environmental microbiologists through methods that reproduce the natural environment of microorganisms to promote their growth.[32] For about 9 years now, culturomics a high throughput culture method has been developed and has allowed and has allowed to widen in a condidable way the repertoire of

cultured bacteria known to man.[33, 34] In this study, we focused particularly on the skin microbiota of African subjects.

In order to expand the repertoire of bacteria associated with the skin of the palm of the hand microbiome, we propose to apply the culturomic approach to 402 skin swab samples from healthy individuals residing in Dielmo and Ndiop, two neighbouring villages in Senegal.

## Material and Methods

### Samples collection

A total of 402 samples of skin swabs from the palm of hand were collected in from January to December 2016 from healthy subjects from Dielmo and Ndiop in rural Senegal. The bacteriological swabs are inoculated directly on the agar plates on site at the laboratory at Ndiop. These inoculated media will be incubated for 24 hours at 37°C. The bacterial colonies obtained are identified using the MALDI-TOF type mass spectrometer installed at the main hospital in Dakar. In addition, pathogens not identified by MALDI-TOF are transferred to the Marseille laboratory for molecular identification using standard PCR amplification of the 16s RNA gene followed by sequence. They were treated as soon as they arrived in Marseille in the culturomic laboratory, then stored at -80°C.

### Ethics statement

Before inclusion in the study, written informed consent was required from all adult participants (≥ 18 years) and from parents or legal guardians of minors (≤ 18 years). An information document that clearly explains the risks and benefits associated with the participation to the study was handed over to each patient. This sheet states the reasons and the purpose of the sampling in the presence of a parent / guardian or guarantor. The consent form stipulates that

samples will not be used in the future in other studies not related with the present work without preliminary agreement the participant and the Senegalese national Ethics Committee. The study was approved by the national Ethics Committee of Senegal (N° 53 / MSAS / DPRS / CNERS du 31 mars 2015) and consent was obtained from each individual.

## Culturomics

### Culture media

As a part of culturomics study, the same procedure developed by Lagier *et al*[35] was used. 100µl of each suspension of skin sample was diluted with 900 µl of Dulbecco's Phosphate-Buffered Saline (DPBS) for bacterial culture. $1/10^{th}$ and $1/100^{th}$ dilutions were performed. A total volume of 50 µl of each dilution was used for culturing on directly on Chapman mannitol agar (for selective isolation, research and enumeration of pathogenic bacteria of the genus *Staphylococcus*) was used, followed by Columbia agar with Colistin and Nalidixic Acid (CAN) and 5% sheep blood (for the selective isolation of *Streptococcus* bacteria) and R-medium for the culture of both aerobic and anaerobic bacteria as previously reported.[36] These seeded media are incubated for 24 hours at 37 ° C.

### Identification of strain by MALDI-TOF MS

Bacterial colonies were identified using Matrix Assisted Laser Desorption/ionization Time-Of-Flight (MALDI-TOF) mass spectrometry (BrukerDaltonics, Germany). Each deposit was covered with 2 mL of a matrix solution (saturated α-cyano acid-4-hydroxycinnamic in 50% acetonitrile and 2.5% trifluoroacetic acid). This analysis was performed using MicroFlex mass spectrometer (Bruker Daltonics, Leipzig, Germany) according to the manufacturer's recommendations. The acquired spectrum was then loaded into the MALDI Biotyper Software (Bruker Daltonics) and analyzed, by using the standard pattern-matching algorithm, which

compared the spectrum acquired with that present in the library (Bruker database and ours, constantly updated). Score values of ≥1.7 but <2 indicated identification beyond the genus level, and score values of ≥2.0 indicated identification at the species level. Scores of <1.7 were interpreted as not relevant. An isolate was labeled as correctly identified at the species level when at least one of the colonies spectra had a score ≥ 1.9 and one other of the colonies spectra had a score ≥ 1.7.[37] When the strain remained unrecognized, the 16S RNA gene was sequenced as previously described.[38]

***16s rRNA sequencing***

Sequencing of 16S rRNA gene was performed for unidentified colonies. For DNA extraction, we used the EZ1 DNA Tissue Kit using Biorobot EZ1 Advanced XL (Qiagen, Courtaboeuf, France). The amplification of the 16s rRNA gene was carried out using PCR technology and universal primers FD1 and RP2[39] (Eurogentec, Angers, France). Afterwards, the purification, sequencing, and assembly of the amplified products were realized as previously described.[38] Sequences of 16S rRNA genes were confronted with those which are available in GenBank by BLASTn (http://www.ncbi.nlm.nih.gov/genbank/). A threshold similarity value of >98.7% was chosen for identification at the species level. Below this value, a new species was suspected, and the isolate was described using taxonogenomics.[40, 41]

## Results

A total of 13,927 bacterial colonies were isolated and tested by MALDI-TOF mass spectrometry yielding 176 different bacterial species “Data in S1 File”. Forty-two colonies were unidentified by MALDI-TOF and were then analyzed by 16S rDNA sequencing “Data in S2 File” which identified 39 bacterial species. Of these, 7 had 16S rDNA similarity < 98.5% with

158 the nearest neighbour and were therefore sequenced and considered a new bacterial species:
159 *Citricoccus massiliensis,*[42] *Lysinibacillus timonensis, Microbacterium timonense, Erwinia*
160 *mediterraneensis,*[43] *Corynebacterium senegalense, Arthrobacter seneglensis, Psychrobacter*
161 *timonensis.*[44]
162 These 176 bacterial species "Data in S2 File" belong mainly to *Firmicutes* (75/176;
163 42.61%), but three other bacterial phyla have been found: *Actinobacteria* (71/176; 40.34%),
164 *Proteobacteria* (30/176; 17.04%) and *Bacteroidetes* (1/176; 0.57%) "Fig 1". Among the two
165 most represented phyla in the palm of hand, 53 genera were identified, of which the most
166 represented were *Staphylococcus* (11.86%) followed by *Microbacterium* (7.90%) "Data in S1
167 File", "Fig 2". The aerotolerant were about 175 species (99.43%) "Fig 1", "Data in S1 File".
168 Most of these bacteria (59 species; 33.33%) were first isolated from the human body "Fig 1",
169 "Data in S3 File".
170 Other microorganisms were isolated for the first time from the environment (55 species;
171 31.25%) and from animals (33 species; 18.75%), plants (15 species; 8.52%) "Fig 1", "Data in S3
172 File". 72 species (72/176 species; 40.91%) were first cultured at 37◦C, and 98 species (98/176
173 species; 55.68%) were cultured at temperatures below 37◦C (i.e., 28, 30 and 35◦C). Of the 176
174 bacteria isolated, 9, 65% were already known to the skin microbiota (17/176 species), 24% were
175 already isolated in other sites of the human body and 4% are new species of culturomics isolated
176 during this or other projects. The 62% were not known in humans. They come from the
177 environment, animals or plants "Fig 1", "Data in S3 File".
178 Pathogenicity is represented as previously mentioned according to its risk group. Risk
179 group 1 category refers to no or low individual and community risk, risk group 2 refers to species
180 with moderate individual risk and a low community risk, and unclassified refers to species that
181 were not found in the risk group database. Of the 176 isolated bacteria, 64% (113/176) are risk

group 1. 25% (45/176) and 11% (19/176) are unclassified “Fig 1”, “Data in S3 File”.

These phyla include 53 different genera. *Staphylococcus* and *Microbacterium* (35 species, 19.77% were those containing the largest number of species “Fig 2”, “Data in S1 File”. These 53 genera are divided into 27 families, of which *Micrococcaceae* (29 species, 16.38%) was the most represented “Fig 2”, “Data in S1 File”.

Compared to the recent repertoire associated with skin, only 26.14% (46/176) of the bacterial species have already been isolated from skin samples. Our study thus increases the repertoire of bacterial species isolated from skin samples by 11.45% (60/524) per culture. According to Bilen and colleagues,[45] among these 60 bacteria that we added to the skin microbiota 7 were new bacterial taxa “Fig 3”.

We isolated 176 species including 7 new species, 64 species isolated for the first time in humans and 60 species known in humans but isolated for the first time from the skin. We made a comparison between the previously established repertoire of microorganisms from the gut[46] updated by those added by Bilen et al.,[45] from urine,[47] vagina,[48] Milk,[49] respiratory tract[50] and the established repertoire from Skin updated by the results of this study.

The Venn diagram “Fig 4” showed that the skin shared 94 bacterial species with the human gut. This finding can be explained by the fact that the intestine hosts the richest community among human anatomical sites.[51]

Finally, the culturomic approach applied to skin specimens allowed us to capture 33.58% (176/524) of the prokaryotes inhabiting the “Data in S1 File”. We also increased the prokaryotic repertoire of the skin by 11.45% and discovered 7 new taxa. “Data in S1 File”

**Discussion**

Nevertheless, to date, microbiome studies have mostly focused on populations living in developed countries. Of the few studies that have included a more diverse array of populations,

most have contrasted urban populations in highly developed countries to populations in developing countries,[17, 52] or populations having both contrasted diet and occupying distinct climates.[53] Such designs do not allow the respective influences of the many factors coupled to geography such as diet, climate, hygiene, parasitism, and host genetics on microbiome variation to be disentangled. While some specific changes in microbial communities have been linked to components of human dietary regimes,[54] urbanization levels,[55] hygiene practices,[17] and the use of antibiotics[56] the effect of other environmental or hostrelated factors is not clear. Notably, we do not know the extent to which the observed loss of microbial diversity of the human gut microbiome in urban industrialized populations[53] is attributable to their dietary specialization, differences in pathogen/parasite exposure, or other environmental factors.[57]

Molecular approaches examining bacterial diversity have underlined the concept that the skin microbiota is dependent on the body site and that caution should be taken when selecting and comparing sites for skin microbiome studies. Grice et al.  have demonstrated that colonization of bacteria is dependent on the physiology of the skin site, with specific bacteria being associated with moist, dry and sebaceous microenvironments.[58] *Propionibacterium* spp. are the dominant organisms in these and other sebaceous areas, which confirms classical microbiological studies that describe *Propionibacterium* spp. as lipophilic residents of the pilosebaceous unit. Microbial transplant experiments suggest that the microenvironment of sebaceous areas such as the forehead) is a stronger force in determining microbial colonization than the microenvironment of dry areas such as the forearm.[15] Metagenomic analysis revealed that moist areas are heavily colonized by *Staphylococcus* and *Corynebacterium spp*,[15, 25] consistent with culture data suggesting that these organisms prefer areas of high humidity.

Like the digestive microbiota, the composition of the skin microbiota can be related to the environment. Differences in the composition of the microbiota can be related to multiple factors

depending on genetics, lifestyle, diet, hygiene and the environment.[59] Recent studies carried out in Africa have shown that modifications of the skin microbiota are linked to geographical origin.[60] In general, a higher diversity of the digestive microbiota is observed in African subjects and more generally in populations living in intertropical areas.[61]

A study of the digestive microbiota in populations of hunters and gatherers of Hadza in Tanzania had observed a high abundance of *Spirochaetes*, *Prevotella*, *Clostridiales*, *Ruminobacter*, *Proteobacteria*, etc., compares to that of urban populations where *'Firmicutes*, *Bacteroides* and *Bifidobacterium* predominate. The results of this work showed a discrepancy in the *Prevotella* and *Bacteroides* report, which could be related to the diet.[62, 63]

A study of breast milk in Africa shows a predominance of *Staphylococcus epidermidis*, *Streptococcus* spp. and LAB species, the main groups of bacteria isolated by culture. These results are similar to those of studies in European women in which staphylococci, enterococci, lactococci and LAB (lactic acid bacteria) species were the most common bacterial species isolated from breast milk.[28, 29]

These results suggest that environmental factors may not have a large influence on the bacterial composition of human milk. Previous studies in West Africa have also revealed similarities in the concentration of breast milk fat between Gambian and British mothers, despite significant differences in the nutritional components of their diets[64] *S. aureus*, *S. hominis* and *S. parasanguis* were particularly more common in infants aged 14 days (first lactation period) than in older infants, highlighting the specific characteristics of the composition of human milk in the first weeks of breastfeeding. These results highlight the difference in certain frequencies of bacterial counts according to the lactation period.[31]

Although partial contamination from skin microbes occurs, the presence of strictly anaerobic species such as *Bifidobacterium*, *Clostridium*, and some *Bacteroides* spp., which are

absent in the skin microbiota, supports that breast milk hosts a unique microbiome.[65, 66]

To our knowledge this study is the first Culturomics study on healthy skin in Africa and allowed us to capture 33.58% (176/524) of the prokaryotes inhabiting the skin. We also increased the prokaryotic repertoire of the skin by 11.45% and discovered 7 new taxa by expanding the repertoire of known microorganisms in the skin microbiota and collecting viable strains of these cultured microorganisms, our work paves the way for clinical studies. Except for an Egyptian study that found four predominant phyla, namely: *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Deinococcus-Thermus*,[67] the absence of studies of the cutaneous microbiota in Africa is a limitation for this study as the results cannot be compared to those of similar studies. In Egypt researchers found a greater genus diversity in *Proteobacteria* with *Klebsiella*, *Bacillus*, *Pseudomonas* and *Escherichia* being predominant. The difference with the results of our study could be due to Egyptian geographical, environmental, etc. factors which are not the same as in Senegal. Indeed, many common skin disorders are believed to have an underlying microbial contribution because clinical improvement is observed with antimicrobial treatments. The different ways in which a skin disease can be associated with a specific organism can be illustrated with three cases: first, a skin disorder with a correlation to the microbiota; second, a skin disorder with a currently unidentified microbial component; and third, a skin commensal that can become invasive to cause an infection.[58]

The composition of the skin microbiota results from a balance between local conditions and the metabolic properties of its microorganisms.[68] Resident or transitory, the microbiota of the skin varies quantitatively and qualitatively from person to person according to age, sex, location, immune system and certain physico-chemical factors such as humidity, pH and temperature.[69] The resident flora consists of commensal germs, which colonize the body without causing disease. The composition and distribution of this flora is relatively stable. She is able to

spontaneously restore herself after a disruption. In particular, it plays an important role in resistance to colonization by other potentially pathogenic microorganisms.[70]

Gram+ species with two main families: staphylococci and aerobic coryneform bacteria (*Corynebacterium spp.*) and anaerobic (*Propionibacterium spp*.) dominate this resident flora. Coagulase-negative staphylococci are the most common species found in normal skin flora, three species predominate: *S. epidermidis*, which can be isolated throughout the skin, constitutes more than 90% of the aerobic resident flora present on the stratum corneum. *S. hominis* which is frequently isolated from the axillary cavity, inguinal cavity and perineum; *S. haemolyticus* which is mainly found in the arms, legs and interdigital spaces. *Corynebacteriform* organisms include *Corynebacteria* and bacteria of the genus *Brevibacterium*, *Propionibacteria*, and microscopic shells. The only resident Gram negative bacteria in the skin are of the genus *Acinetobacter*.[70]

The culturomics approach to characterizing microbial diversity has significantly changed our view of the skin microbiota, subsequently raising many important questions about the host-microorganism relationship and its relevance to skin diseases. Although it is now clear that a large proportion of the skin microbiota consists of *Staphylococcus* and *Propionibacterium* spp, little is understood about the rare or transient organisms that make up the remainder. The factors that cause variation in these organisms are unknown, nor how or how fluctuation is associated with skin diseases. Metagenomic analysis to elucidate the microbial gene pool and its functions should lead to a better understanding of these issues. The pathogenesis of numerous dermatologic conditions remain unexplained. The hypothesis of infection has been proposed for diseases such as scleroderma, psoriasis, eczema, seborrheic dermatitis, dyshidrosis, acne, and rosacea. Is it possible that specific microbes, or changes in the microbial community on the skin, initiate or sustain some of these diseases?

Microbiotes present on the skin may be transient or resident, the latter are considered

members of the normal commensal skin microbiota that is homeostasis with the host, while the former are microbes that live temporarily on the skin[71] Disruption of the homeostatic relationship between the host and the microbiota could also be a determining factor in inflammatory skin diseases such as atopic dermatitis and psoriasis.[72] The cutaneous innate immune response in the patient with atopic dermatis). The seasonality of skin diseases suggests that skin microbiota might change according to climate and time. Increases in external temperature and humidity due to clothing are known to increase bacterial loads and variability in species distributions.[73]

**Acknowledgments**

We thank the populations of Dielmo and Ndiop from Senegal in Ouest Africa for their participation and for their continuing collaboration in the study, as well as the investigators. This work has benefited from the French State support, managed by the 'Agence Nationale pour la Recherche' including the "Programme d'Investissement d'avenir" under the reference Méditerranée Infection 10-IAHU-03.

**Figure legends**

**Fig 1.** Proportion of bacterial species from the palm of hand, listed according to their phylum, oxygen tolerance, risk group, Kind of species, biological origin and temperature at first isolation.

**Fig 2.** Most represented taxa from the skin exactly the palm of the hand. (A)Word cloud showing the most represented families from the skin. (B) Word cloud showing the most represented genera from the skin

**Fig 3:** Number of different bacteria isolated during the culturomics studies.

Column “Before culturomics represent results from previously published studies (Unpublish data) while the column and columns “culturomics studies” the result on the current study. The bacterial species are represented in five categories: new species from our study; prokaryotes unknown in humans; prokaryotes unknown in Skin; prokaryotes isolated by our study previously known from skin.

**Figure 4.** Venn diagram showing the shared cultured species between human skin (this study) and gut,[13] urine,[23] vagina,[24] Milk,[25] respiratory tract.[26]

**Supplementary data legend**

**Supplementary data 1 :** Bacteria isolated from swabs

Table showing the bacteria isolated and identified from skin swabs cultured on different media and the results of the taxonomy

**Supplementary data 2 :** Liste of bacteria identified by sequencing

This table lists the 39 bacteria that we were unable to identify by MALDI-TOF. They are part of the 176 bacteria previously isolated following the inoculation of swabs on culture media. Their identifications were made by the technique of sequencing.

**Supplementary data 3 :** List of bacteria isolated from swabs with specific characteristics

This table lists the 176 bacteria isolated with information on risk group; oxygen tolerance: the medium in which they were first isolated; incubation temperature; and whether they are human, skin or environmental bacteria.

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# Partie III

## Portage asymptomatique de certaines bactéries pathogènes détectées par la méthode de qPCR sur la paume des mains des sujets en bonne santé

Avant propos

Les maladies infectieuses sont une cause importante de décès en Afrique subsaharienne et continuent de poser des problèmes majeurs de santé publique dans les pays africains, notamment la pneumonie. Le portage cutané de certaines bactéries pathogènes telles que *S. pneumoniae* est souvent ignoré ou sous-diagnostiqué. Enfin, le mode de transmission de ces infections reste incertain. Dans cette partie du travail de cette thèse, nous avons émis l'hypothèse que la peau pourrait jouer un rôle dans la transmission de ces infections. Nous avons collecté 649 écouvillons effectués au niveau de la paume de la main auprès d'une population saine à Dielmo et Ndiop, dans les zones rurales du Sénégal. Après extraction de l'ADN et contrôle de l'actine (pour contrôler la qualité de l'extraction), qPCR ciblant huit bactéries différentes a été effectuée sur 614 échantillons (nombre de prélèvements avec une bonne qualité d'extraction de l'ADN). Nous avons détecté *Streptococcus pneumoniae* dans 33,06% (203/614), Staphylococcus aureus dans 18,08% (111/614) et *Streptococcus pyogenes* dans 1,95% (12/614) des échantillons. Un portage cutané de *S. pneumoniae* a été détecté dans plus d'un tiers d'une population rurale en Afrique rurale, ce qui souligne la nécessité de développer des programmes de désinfection des mains afin de réduire le portage cutané de ses bactéries pathogènes.

Article 3

# Asymptomatic carriage of Streptococcus pneumoniae detected by qPCR on the palm of hands of populations in rural Senegal

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Publié dans le journal PLOS Neglected Tropical Diseases

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

# Asymptomatic carriage of *Streptococcus pneumoniae* detected by qPCR on the palm of hands of populations in rural Senegal

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**Citation:** Ndiaye C, Bassene H, Lagier J-C, Raoult D, Sokhna C (2018) Asymptomatic carriage of *Streptococcus pneumoniae* detected by qPCR on the palm of hands of populations in rural Senegal. PLoS Negl Trop Dis 12(12): e0006945. https://doi.org/10.1371/journal.pntd.0006945

**Editor:** Katja Fischer, QIMR Berghofer Medical Research Institute, AUSTRALIA

**Received:** July 3, 2018

**Accepted:** October 25, 2018

**Published:** December 10, 2018



**Data Availability Statement:** Data are available from the UMR 257, VITROME of IRD Senegal Institutional Data Access (https://www.mediterranee-infection.com/acces-ressources/donnees-pour-articles/asymptomatic-carriage-of-streptococcus-pneumoniae-detected-by-qpcr-on-the-palm-of-hands-of-populations-in-rural-senegal/). Data are from the Soap Project study whose authors may be contacted at mouhamadou-baba.sow@ird.fr.

## Abstract

Aside from malaria, infectious diseases are an important cause of death in sub-Saharan Africa and continue to pose major public health problems in African countries, notably pneumonia. *Streptococcus pneumoniae* remains the most common bacterial cause of pneumonia in all age groups. The skin is one of the main infection sites followed by the oropharynx. The skin carriage of certain pathogenic bacteria such as *S. pneumoniae* is often ignored or under-diagnosed. Finally, the mode of transmission of these infections remains uncertain. Here, we hypothesized that skin could play a role in the transmission of these infections. We collected 649 cotton swabs from a healthy population in Dielmo and Ndiop, rural Senegal. The sampling was carried out on the palm of the hands. After DNA extraction and actin control, qPCR targeting eight different bacteria was performed on 614 skin samples. We detected *Streptococcus pneumoniae* in 33.06% (203/614), *Staphylococcus aureus* in 18.08% (111/614) and *Streptococcus pyogenes* in 1.95% (12/614) of samples. A skin *S. pneumoniae* carriage was detected in more than a third of a rural population in rural Africa, highlighting the need to develop hand disinfection programs in order to reduce the burden of infections.

## Author summary

Infectious diseases are one of the leading causes of morbidity and mortality in the world. They kill nearly 17 million people worldwide each year, mainly in developing countries. They are transmitted through four main channels: air, oral, parenteral and contact. The prevention of infectious diseases requires an understanding of the population's way of life and the knowledge of pathogenic microorganisms in circulation. Many diseases are transmitted through contact with soiled hands. This study allowed us to explore the pathogenic bacteria carriage on the skin in a rural population, following skin swabs made on the palms of the hands. Previous studies have shown that hands play an important role in the

**Funding:** IHU Méditerranée Infection financed the study but the funds were managed by IRD. All requests for information related to this financing can be addressed to Michel Drancourt (michel.drancourt@univ-amu.fr). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors have declared that no competing interests exist.

transmission and the prevention of infectious diseases such as diarrhea and pneumonia. One of the main results is the detection in high proportion of pathogenic bacteria such as *Staphylococcus aureus* and especially *Streptococcus pneumoniae*. Life in rural areas exposes the population to a lot of pathogenic microorganisms. These results could be used to implement prevention strategies against certain infectious diseases, by raising awareness of the importance of body hygiene and specifically hands hygiene, in order to improve the health of the population.

## Introduction

Infectious diseases are the most important cause of death in sub-Saharan Africa [1, 2] and continue to pose major public health problems in African countries. Globally and collectively they account for 20% of the mortality in all age groups (and 33% of the mortality in the least developed countries) and 50% of infant mortality [3]. A study performed in Karachi, Pakistan found that 41% of deaths of children under 5 year were due to diarrhea and 15% to acute respiratory infections which include pneumonia [4]. The pathogenic role of *Streptococcus pneumoniae* in pneumonia, otitis media, bacteremia and meningitis is undisputed. However, its isolation on the skin is an unusual discovery with a difficult clinical interpretation [5] which can range from simple colonization in immunocompetent hosts to severe infection in patients with different underlying conditions [5–6]. In a study performed in 2014, Fenollar et *al*. reported that some bacteria that cause fever in Africa such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae* are neglected in Senegal [7]. The monitoring of the carriage of these bacteria is important for several reasons. First, colonization in healthy individuals is a prerequisite for developing invasive and non-invasive diseases, and reduced colonization has been correlated with decreased pneumococcal and staphylococcal infection rates [8–9]. Second, healthy carriers serve as reservoirs for *S. aureus* and *S. pneumoniae* transmission to others in the community and in the hospital [10–11]. *S. pneumoniae* is one of the major pathogens infecting humans worldwide and is the most common cause of community-acquired bacterial pneumonia and otitis media, but can also give rise to severe cases of meningitis and sepsis [12]. Approximately 1.6 million people die each year from pneumococcal diseases [12]. The most frequently bacteria isolated in acute respiratory infections are *S. pneumoniae* and *Haemophilus influenzae*, which can occur secondarily following primary infection due to viral pathogens. Despite causing severe diseases, the asymptomatic carriage of *S. pneumoniae* in the nose, nasopharynx and throat was also reported. The isolation rates of *S. pneumoniae* obtained by nasal and nasopharyngeal (NP) sampling are similar in children, but higher than that of oropharyngeal sampling [13,14]. Its prevalence in nasopharyngeal samples varies from 7 to 99% and depends on the age, health, and socioeconomic status of the study population [15]. In Senegal, few studies on the viral and bacterial etiology of respiratory tract infections are available in pediatric settings [16]**.** Most of the publications on the prevalence of *S. pneumoniae* in NP samples concerned young children. Indeed, in 2003, Echave et *al*. reported a prevalence rate of 56% in Senegal [17]. However, Baylet et *al*., in 1983, reported higher NP carriage prevalence rates in rural (77%) and urban (69%) areas in Senegal and other African countries [18].The study conducted by Mediannikov et *al*.in 2014, in Senegal revealed a skin carriage of *S. aureus* (21.7% %), *S. pneumoniae* (5%) and *S. pyogenes* (5%) on the skin of healthy people leaving in rural areas [19].

Here, we studied the skin carriage of major pathogenic bacteria such as *S. pneumoniae*, *S. aureus* and *S. pyogenes* in the populations of Dielmo and Ndiop, two rural villages. In an

ancillary study, we tested the skin carriage of *R. felis*, *B. crocidurae*, *T. whipplei*, *B. quintana* and *C. burnetii* [19–22] which have been described as causes of fever in this rural area.

## Materials and method

### Ethics statement

Before inclusion in the study, written informed consent was required from all adult participants ($\geq$ 18 years) and from parents or legal guardians of minors ($\leq$ 18 years). An information document that clearly explains the risks and benefits associated with the participation to the study was handed over to each patient. This sheet states the reasons and the purpose of the sampling in the presence of a parent / guardian or guarantor. The consent form stipulates that samples will not be used in the future in other studies not related with the present work without preliminary agreement of the Senegalese national Ethics Committee. Consent was obtained from each individual, and the study was approved by the national Ethics Committee of Senegal (N° 53 / MSAS / DPRS / CNERS du 31 mars 2015).

### Study site

Dielmo and Ndiop are two villages located about 280 km Southeast of Dakar, near the Gambian border in an area of Sudan-type savannah. About 700 inhabitants have been included in an epidemiological study of malaria since 1990 and monitored daily for fever and illness; the detection of cases is both active and passive. The geographical and epidemiological characteristics of the Dielmo village have been previously described in detail elsewhere [23,24], most of the houses are built in the traditional style with mud walls and thatched roofs. The main source of drinking water of the population is underground water.

### Study population

The study population consisted of people residing in the two villages, participating in the epidemiological follow-up of Dielmo and Ndiop, adhering to the principle of the project and having given their consent to participate in the study and thus provide a swab. Sampling was carried out on the palm of the hands (the right and left palm of unwashed hands) of a healthy population, all categories of age were included. It was performed in January in order to make an inventory of the skin carriage of the targeted pathogens. In addition, the sampling was done at the same location in each study participant.

### Swabs and samples analysis

Swabs are performed on the hands, after moistening the swab with sterile physiological serum. The swabs obtained are immersed in an individual tube containing 600 μl of 1X Phosphate Buffer Saline (PBS, OXOID LIMITED, HAMPSHIRE, ENGLAND). Once impregnated, the swab is pressed against the edges of the tube to release the sample, then 200 μl of the swab suspension was taken for DNA extraction using the CTAB 2% method.

### DNA extraction

To extract the DNA, 200μl of bacterial suspension from the swab was mixed with 180μl of 2% Cetyl Trimethyl Ammonium Bromide (CTAB) [25]. The mixture was incubated in a water bath at 65˚C for 1 hour. Two hundred microliters of chloroform were added to the mixture, and the supernatant was recovered after centrifugation at 12,000 rpm for 5 min. The nucleic acids were precipitated by 200 μl of isopropanol after15 min of centrifugation at 12,000 rpm. The pellet was then dried in a speed vac for 3–4 min and resuspended in 200 μl RNase-free

water. The DNA solution was stored in the refrigerator at 8˚C until further use and the PCR was done 24 hours after DNA extraction, as DNA cannot deteriorate after only 24 hours of storage. After the PCR, the DNA was conserved at -20˚C.

### DNA amplification

Except for *Bartonella*, bacteria were detected using a first intent PCR, when a specimen was tested positive in the first assay, the result was confirmed by a second quantitative PCR. A positive sample was defined as 2 positive quantitative PCR results in assays targeting 2 different repeated DNA sequences. We performed all PCR reactions in a CFX 96 thermal cycler (Biorad). Each reaction was performed at a final volume of 20 μl, containing, 10μl of polymerase TAKYON, 1μl of each primer, 1μl of probe, 2μl of RNase-free water and 5μl DNA. A positive and negative control was included in each experiment. DNA extracted from the swabbing of a healthy person in Dakar was used as negative control. The positive control consists of a suspension made from a swabbing of healthy person, in which bacterial cultures were added. The strains used as positive control are available on the « Collection de Souches de l'Unité des Rickettsies (CSUR, WDCM 875) » under the accession number: *S. pneumoniae* CSURP5700, *S. aureus* CSURP2200 and *S. pyogenes* CSURP6897. For each species, about ten colonies were suspended in 200μl of PBS and DNA was extracted as mentioned above.

Molecular identification by qPCR involved pathogenic bacteria such as *S. pneumoniae*, *S. aureus*, *R. felis*, *B. crocidurae*, *T. whipplei*, *B. quintana*, *S. pyogenes* and *C. burnetii* (Table 1).

The quality of the extraction was measured by actin (the b-actin gene amplification by quantitative PCR confirmed the quality of the extracted DNA). Any sample with a Ct number (cycle threshold) that did not exceed 35 was considered positive. This number corresponds to the ability to reveal 10–20 copies of bacterial DNA [20,21].

### Statistical analysis

Data were analyzed using Open Epi, version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Non-parametric values were compared using a $X2$ test. Statistical significance was defined as $p < 0.05$.

## Results

A total of 649 samples was collected, 246 in Dielmo and 403 in Ndiop.

Among the 649 samples tested, 614 (94.61%) were actin-positive and 35(5.4%) were actin-negative.

### Prevalence of bacteria in Dielmo and Ndiop by qPCR

We detected *S. pneumoniae* in 33.06% (203/614) individuals, *S. aureus* in 18.08% (111/614) individuals and *S. pyogenes* in 1.95% (12/614) individuals.

We also detected *C. burnetii* in 13.35% (82/614) individuals, *B. crocidurae* in 3.42% (21/614) individuals. However, we didn't find any case of *T. whipplei*, *B. quintana* or *R. felis* (Table 2).

We observed that *S. pneumoniae* and *S. aureus* were the two predominantly isolated bacteria out of eight targeted bacteria, and tried to see their incidence according to age and sex.

### Prevalence of *S. pneumoniae* and *S. aureus* by age group in Dielmo and Ndiop

In Dielmo and Ndiop *S. pneumoniae* DNA was detected in all age groups with percentages greater than 20%. *S. pneumoniae* DNA was mostly detected in the youngest age groups of 0–5

**Table 1. Target sequences, primers and probes used in a study.**

| Targeted Organism | Targeted gène | Name | Sequences | References |
|---|---|---|---|---|
| **All Rickettsia except *R. typhi/ R. prowasekii*** | RC0338 | 1029-F1 | GAM AAA TGA ATT ATA TAC GCC GCA AA | [26] |
| | | 1029-R1 | ATT ATT KCC AAA TAT TCG TCC TGT AC | |
| | | Rick1029_MBP | 6FAM- CGG CAG GTA AGK ATG CTA CTC AAG ATA A-TAMRA | |
| ***R. felis*** | Biotin syntase | R_fel0527_F | ATG TTC GGG CTT CCG GTA TG | [26] |
| | | R_fel0527_R | CCG ATT CAG CAG GTT CTT CAA | |
| | | R_fel0527_P | 6FAM- GCT GCG GCG GTA TTT TAG GAA TGG G -TAMRA | |
| **All *Bartonella*** | ITS | Barto_ ITS3_ F | GAT GCC GGG GAA GGT TTT C | [27] |
| | | Barto_ ITS3_ R | GCC TGG GAG GAC TTG AA CCT | |
| | | Barto _ITS3 _P | 6FAM- GCG CGC GCT TGA TAA GCG TG -TAMRA | |
| **All *Borrélia*** | 16S | Bor_16S_3_F | AGC CTT TAA AGC TTC GCT TGT AG | [28] |
| | | Bor_16S_3_R | GCC TCC CGT AGG AGT CTG G | |
| | | Bor_16S_3_P | 6FAM-CCG GCC TGA GAG GGT GAA CGG-TAMRA | |
| ***B. crocidurae*** | glpQ | Borcro_glpQ_F | CCT TGG ATA CCC CAA ATC ATC | [29] |
| | | Borcro_glpQ_R | GGC AAT GCA TCA ATT CTA AAC | |
| | | Brocro_glpQ_MGB_P | 6FAM-ATG GAC AAA TGA CAG GTC TTAC-MGB | |
| ***C. burnetii*** | IS1111A | Coxbur_IS1111_0706_F | CAA GAA ACG TAT CGC TGT GGC | [20] |
| | | Coxbur_IS1111_0706_R | CAC AGA GCC ACC GTA TGA ATC | |
| | | Coxbur_IS1111_0706_P | 6FAM-CCG AGT TCG AAA CAA TGA GGG CTG-TAMRA | |
| | Hyp.Protein | Coxbur_IS30A_3_F | CGC TGA CCT ACA GAA ATA TGT CC | [20] |
| | | Coxbur_IS30A_3_R | GGG GTA AGT AAA TAA TAC CTT CTG G | |
| | | Coxbur_IS30A_3_P | 6-FAM- CAT GAA GCG ATT TAT CAA TAC GTG TAT GC-TAMRA | |
| ***S. aureus*** | NucA | Saur__NucA__F | TTG ATA CGC CAG AAA CGG TG | [19] |
| | | Saur__NucA__R | TGA TGC TTC TTT GCC AAA TGG | |
| | | Saur__NucA_MGB_P | 6FAM- AAC CGA ATA CGC CTG TAC -MGB | |
| | *Amidohydrolase* | Saur_F | CCT CGA CAG GTA ACG CAT CA | [19] |
| | | Saur_R | AAA CTC CTA TCG GCC GCA AT | |
| | | Saur_P | 6FAM-TGC AAT GGT AGG TCC TGT GCC CA | |
| ***S. pyogenes*** | hypothetical | Spyo_hypp_F | ACA GGA ACT AAT ACT GAT TGG AAA GG | [19] |
| | | Spyo_hypp_R | TGT AAA GTG AAA ATA GCA GCT CTA GCA | |
| | | Spyo_hypp_P | 6FAM- AAAATGTTGTGTTTTAGGCACTGGCGG-TAMRA | |
| | MipB | Spyo_mipB_F | CCA TAC GGT TAT AGT AAG GAG CCA AA | [19] |
| | | Spyo_mipB_R | GGC TAT CAC ATC ACA GCA ACC | |
| | | Spyo_mipB_P | 6FAM- TCAGCGCCAGCTTCAATGGC- TAMRA | |
| ***S. pneumoniae*** | plyN | Pneumo_plyN_F | GCG ATA GCT TTC TCC AAG TGG | [19] |
| | | Pneumo_plyN_R | TTA GCC AAC AAA TCG TTT ACC G | |
| | | Pneumo_plyN_P | 6FAM-CCC AGC AAT TCA AGT GTT CGC CGA-TAMRA | |
| | lyt A | Pneumo_lytA_F | CCT GTA GCC ATT TCG CCT GA | [19] |
| | | Pneumo_lytA_R | GAC CGC TGG AGG AAG CAC A | |
| | | Pneumo_lytA_P | 6-FAM- AGA CGG CAA CTG GTA CTG GTT CGA CAA-TAMRA | |
| ***T.whipplei*** | WiSP family protein (WHI2) | T_whi2_F | TGA GGA TGT ATC TGT GTA TGG GAC A | [21] |
| | | T_whi2_R | TCC TGT TAC AAG CAG TAC AAA ACA AA | |
| | | T_whi2_P | 6FAM- GAG AGA TGG GGT GCA GGA CAG GG-TAMRA | |
| | WiSP family protein (WHI3) | T_whi3_F | TTG TGT ATT TGG TAT TAG ATG AAA CAG | [21] |
| | | T_whi3_R | CCC TAC AAT ATG AAA CAG CCT TTG | |
| | | T_whi3_P | 6FAM- GGG ATA GAG CAG GAG GTG TCT GTC TGG-TAMRA | |

https://doi.org/10.1371/journal.pntd.0006945.t001

**Table 2. Results of prevalence of pathogenic bacteria in Dielmo and Ndiop.**

| Bacterium identified | Incidences (%) |
|---|---|
| *Streptococcus pneumoniae* | 33.06% (203/614) |
| *Coxiella burnetii* | 13.35% (82/614) |
| *Staphylococcus aureus* | 18.08% (111/614) |
| *Borrelia crocidurae* | 3.42% (21/614) |
| *Streptococcus pyogenes* | 1.95% (12/614) |

https://doi.org/10.1371/journal.pntd.0006945.t002

and 5–10 years with respectively 39.40% (47/119) and 47.93% (58/121). The lowest rates, 17.02% (8/47) was obtained in the age groups of 45–60 years. *S. aureus* was detected mostly in the 5–10, 10–15 and 15–30 years age groups with 19.83% (24/121), 22.89% (19/83) and 19.55% (26/133), respectively, and in a minority in the +60-years groups, with 8.82% (3/34). Significant difference was noticed only in the 0–5 and 5–10 years age groups (Table 3).

## Prevalence of *S. pneumoniae* and *S. aureus* by sex in Dielmo and Ndiop

Studies on these samples from 614 villagers in Dielmo and Ndiop showed a skin carriage in 46.74% (115 /246) of men and 23.91% (88/368) of women for *S. pneumoniae*, in 14.22% (35/246) of men and 20.65% (76/368) of women for *S. aureus*. Statistical analysis showed a significant difference between men and women for *S. pneumoniae* ($p < 0.0000001$) but for *S. aureus* no significant difference between men and women was observed ($p = 0.05485$) (Table 4).

## Discussion

In our study, we attempted to identify the prevalence of skin carriage of specific bacteria in a generally healthy rural population in Senegal. We are confident of our results because the validity of the data reported in this study is based on strict experimental procedures and positive and negative controls. The sampling was correct because 95% of samples were actin positive. Molecular analysis were carried out in two villages Dielmo and Ndiop. The most common pathogens detected were *S. pneumoniae* and *S. aureus*. They represented 51.14% of the pathogenic bacteria identified on the skin. To our knowledge, this study is the first attempt to investigate the presence of *S. pneumoniae* and *S. aureus* in the skin of asymptomatic peoples in Africa. We are also considering whether there is a link between identified pathogens and skin infections; pneumonia and respiratory infections.

It is important to note that the differences in bacterial diversity on the skin varies from one site to another at the inter and intrapersonal level, for example, the bacterial diversity of the forehead is lower than the diversity of the palm in each person, and this is also true for the forehead versus forearm communities [30]. For our study, we found it more interesting to swab

**Table 3. Results of prevalence of pathogenic bacteria in Dielmo and Ndiop by Age-group.**

| Age group (years) | *S. pneumoniae* | *S. aureus* | *P* & $\chi^2$ |
|---|---|---|---|
| **0–5** | 39.40% (47/119) | 15.96% (19/119) | $P = 0.0000001$ ($\chi^2 = 48.11$, df = 1) |
| **5–10** | 47.93% (58/121) | 19.83% (24/121) | $P = 0.000007$ ($\chi^2 = 20.1$, df = 1) |
| **10–15** | 37.34% (31/83) | 22.89% (19/83) | $P = 0.063$ ($\chi^2 = 3.5$, df = 1) |
| **15–30** | 23.30% (31/133) | 19.55% (26/133) | $P = 0.5$ ($\chi^2 = 0.36$, df = 1) |
| **30–45** | 25.97% (20/77) | 15.58%(12/77) | $P = 0.16$ ($\chi^2 = 1.93$, df = 1) |
| **45–60** | 17.02% (8/47) | 17.02% (8/47) | $P = 0.78$ ($\chi^2 = 0.07$, df = 1) |
| **+60** | 23.52% (8/34) | 8.82% (3/34) | $P = 0.18$ ($\chi^2 = 1.7$, df = 1) |

https://doi.org/10.1371/journal.pntd.0006945.t003

**Table 4. Results of prevalence of pathogenic bacteria in Dielmo and Ndiop by sex.**

| | Men | Women | *P* & $\chi^2$ |
|---|---|---|---|
| ***S. pneumoniae*** | 46.74% (115 /246) | 23.91% (88/368) | $p < 0.0000001$ ($\chi^2 = 33.71$, df = 1) |
| ***S. aureus*** | 14.22% (35/246) | 20.65% (76/368) | $p = 0.05485$ ($\chi^2 = 3.687$, df = 1) |

https://doi.org/10.1371/journal.pntd.0006945.t004

the palms of the hands because we believe that it is the most likely means of bacterial transmission, through salutation, food, etc. Next to *S. pneumoniae* and *S. aureus* we detected the DNA of *B. crocidurae* and *C. burnetii*, but their known mode of transmission is not through the palms of the hands. *S. pneumoniae* was the most prevalent bacteria detected on the on skin. This bacterium is one of the leading causes of pneumonia in children under five years of age in Senegal [31]. *S. aureus* was the second most prevalent bacteria detected. The results obtained on the prevalence of this bacterium are in line with those of Mediannikov et *al.*, 2014 which detected 21.7% (13/60) of *S. aureus* DNA on the forearm of asymptomatic populations residing in the same area [19]. We detected also *S. pyogenes* in the skin swabs with a smaller proportion. This rate was lower than that previously reported by Mediannikov et *al.*, 2014, which showed the presence of this bacterium on the forearm of healthy populations (intact skin swabs), with a prevalence of 5% (3/60) on the same study area [19]. This difference could be explained by seasonal variation, being more common in dry than wet seasons in monsoonal climates [32]. Crowding and poor hygiene therefore increase the chance of the transmission of *S. pyogenes*. Also, skin infections are more frequent and are a more important cause of morbidity in overcrowded communities with poor sanitation [33]. In addition, variations in the prevalence of *S. pyogenes* skin infections are related to accessibility to appropriate housing and hygiene. The absence of previous work on the exploration of the skin microbiota in Africa, more specifically in sub-Saharan Africa, is a limitation for our study because these results cannot be compared to those of other countries with different climatic and environmental conditions. Most of the publications on the carriage of *S. pneumoniae* were done on nasopharyngeal swabs and often concerned young children [34].

The highest rates of *S. pneumoniae* and *S. aureus* were detected in the youngest age groups. *S. pneumoniae* was observed in the age groups 0–5 and 5–10 years. The lowest prevalence was found in age groups 45–60 years. These results could be explained by the presence of these bacteria in the environment, and those children who would be much more exposed because they take less care of their hygiene compared to adults who would be much more exposed because they take less care of their hygiene compared to adults. These results are consistent with data from a previous study that found *S. pneumoniae* DNA in 22% (8/36) of skin swabs from the forearm of children in the age group 0–6 years [35]. *S. aureus* was detected mainly in the three youngest age groups 5–10, 10–15 and 15–30 years, and in a minority in the +60-years. Just like *S. pneumoniae*, *S aureus* carriage was affected by age (peak prevalence at youngest age groups). A relationship could be made between *S. pneumoniae* infections in these villagers, a previous study on influenza like illnesses (ILI) had found that the incidence rates differed significantly between age groups, and were highest in the [6–24 month) and [0–6 month) age groups [36]. These results are comparable to ours. Children under five years of age have a higher incidence of *S. pneumoniae*. Finally, in these villages we have set up a field laboratory for the diagnosis of infectious diseases using the molecular biology method [22]. This technical device allows the rapid diagnosis and monitoring of infectious diseases for which laboratory analyses were generally too late to guide therapy [22]. The results show that *S. pneumoniae* infections are mainly localized in children under five years of age.

*S. pneumoniae* causes morbidity and mortality in young children, the elderly and immunodeficient patients [37], but asymptomatic carriage is more common in children. Most

publications on *S. pneumoniae* vaccine research target the youngest age groups. Children are considered an important vector for the spread of this microorganism in the community, and preventing the carriage of pneumococci could therefore reduce the prevalence of infections. Pneumococcal conjugate vaccination protects young children against invasive diseases with *S. pneumoniae* [38]. Researchers have showed that the carriage rate of S. pneumoniae is low in adults compared with children because the prevalence rate, risk factors for carrying and factors promoting the spread of the organism are limited in adults [39]. The main result of our molecular analysis is the carriage of *S. pneumoniae* on the skin in asymptomatic people. *S. pneumoniae* is found all over the world. The incidence of infection is higher in children under 2 years of age and adults over 60 years of age [40]. It belongs to the family *Streptococcaceae*. It is a Gram positive bacterium. There are about 90 serotypes; the capsule surrounding the pneumococcus is the main virulence factor [41]. This bacterium generally colonizes the mucosal surfaces of the nasopharynx and upper respiratory tract, and symptoms of inflammation appear when the bacterium migrates to the sterile parts of the respiratory tract [42]. It is transmitted by infectious cells that can be spread by aerosolized microdroplets sprayed during coughing or sneezing, or by oral contact from one person to another [41]. The nasopharynx is the only documented niche for *S. pneumoniae* in humans, many researchers have speculated on skin colonization following reports of pneumococcal skin and soft tissue infections in adults and children in the absence of prior systemic disease [5,6,43]. However, the rather high detection rates of *S. pneumoniae* in skin samples in this study suggest a possible existence of a true reservoir of this pathogen on the skin. The carriage rates of *S. pneumoniae* reported in previous studies strongly depended on the social, demographic and medical risk factors of the study subjects, as well as the methodological variations in the methodology used. Finally, the sampling sites may vary from one study to another [38]. We have not attempted to associate our prevalence rates of carriage with specific characteristics of the population. In this study, we used qPCR testing to determine the overall prevalence of major pathogens, which had been previously isolated in other types of samples and in the same area, in febrile or healthy subjects [19]. For *S. pneumoniae*, the highest rate of nasopharyngeal colonization has been shown to occur at an early stage of life [11–19]. This corresponds to the low number of *S. pneumonia* carriers in our study, as only adult individuals were sampled.

In this study, we found that 46.74% (115 /246) of men and 23.91% (88/368) of women carried *S. pneumoniae* in their skin samples and statistical analysis showed a significant difference between men and women for the carriage of *S. pneumoniae* ($p < 0.0000001$). Our findings are in line with a previously published study on the carriage of *S. pneumoniae* among older adults in Indonesia [44]. In addition, this is the first time, to our knowledge, that *S. pneumoniae* has been detected in the palm of hands in Senegal. We found that men and women carried *S. aureus*, and statistical analysis showed no significant difference between men and women for the carriage of *S. aureus* ($p = 0.05485$). Our results differ from those of two previous studies that showed that *S. aureus* carriage varies according to the sex and is higher in men [45,46]. To our knowledge, only viral respiratory infections have been studied in Dielmo and Ndiop. Available information shows that respiratory infections due to influenza (flu) viruses are more frequent [47]. From 2012 to 2013, the overall flu incidence density rate was 19.2 per 100 person-years. The flu incidence density rates were significantly different between age groups, the highest being in the [6–24 months) age group (30.3 to 50.7 per 100 person years) [36].

PCR-based techniques suffer from possible biases due to the state of the bacteria (dead bacteria) [43,48]. According to Anna Engelbrektson and *al*., 2010, molecular-based approaches do not distinguish between living bacteria and dead bacteria, so this can lead to the detection of an excessive number of pathogens by qPCR [43,48]. The culture of *S. pneumoniae* from skin

swabs that we made in parallel with the PCR would confirm more accurately the real existence of this bacterium on the skin.

## Conclusion

In our study, we tried to demonstrate the existence of the target pathogens on the skin of people in our generally healthy study population in two villages of rural Senegal and to evaluate their impact in our two study villages using qPCR. Molecular analysis in Dielmo and Ndiop showed a high prevalence of *S. aureus* and *S. pneumoniae* carriage, especially among the youngest age groups. Our results suggest that random samples of skin swabs may contain *S. aureus* and *S. pneumoniae*. In addition, in asymptomatic subjects, we can detect the presence of certain pathogens by qPCR. In rural areas, the economic context and daily activities make this part of the population particularly vulnerable to infectious diseases. These populations live in poverty, the majority of whom are farmers and livestock breeders. As a result, they are less involved in their personal hygiene and food and household hygiene, which would lead to a considerable increase in the prevalence of infectious diseases. Fortunately, previous studies and WHO recommendations showed that body hygiene and more specifically hand hygiene could lead to a significant reduction in the prevalence of these diseases [44,49].

It appears necessary to undertake a so-called "soap project" study in Dielmo and Ndiop villages in order to evaluate the effectiveness of body hygiene in the prevention of infectious diseases.

## Acknowledgments

We thank the populations of Dielmo and Ndiop for their participation and for their continuing collaboration in the study, as well as the investigators. This work has benefited from the French State support, managed by the 'Agence Nationale pour la Recherche' including the "Programme d'Investissement d'avenir" under the reference Méditerranée Infection 10-IAHU-03.

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# Partie IV

## Impact de l’utilisation quotidienne du savon sur les maladies infectieuses respiratoires et sur le portage cutané des bactéries pathogènes

Avant Propos

Cette dernière partie de notre travail à évaluer l'impact de l'évaluation de la promotion de l'hygiène corporelle dans la prévention des maladies infectieuses en milieu communautaire. Les maladies infectieuses sont les principales causes de décès dans le monde et les enfants de moins de cinq ans sont les plus touchés particulièrement dans les pays pauvres. En Afrique, les maladies infectieuses représentent 48% des décès d'enfants de moins de cinq ans dans le monde (UNICEF, 2014). Face à cette gravité de la situation l'Organisation mondiale de la santé (OMS) et l'Unicef à mettre en place le Plan d'action mondial intégré pour la prévention et le contrôle de la pneumonie et de la diarrhée (GAPPD) en 2015 (Qazi *et al*., 2015). Le lavage des mains a été proposé comme une action préventive et peu coûteuse pour prévenir la diarrhée et la pneumonie (Qazi *et al*., 2015). Nous avons réalisé une étude cherchant à évaluer les effets de l'utilisation quotidienne du savon sur l'incidence de la diarrhée, de la fièvre, des infections respiratoires et la prévalence des bactéries pathogènes sur la peau. Nous avons distribué du savon à la population du village de Ndiop (test) pour son hygiène quotidienne, mais pas à la population du village de Dielmo (contrôle). Ensuite nous avons enregistré quotidiennement les événements cliniques dans les deux villages et ont encouragé l'utilisation du savon à Ndiop. 638 personnes ont participé à l'étude. Les taux d'incidence de la toux, de l'écoulement nasal et des fièvres ont diminué de manière significative en 2016 par rapport à 2015, contrairement à ceux de la diarrhée. En 2016, des réductions significatives des taux d'incidence de la toux, de l'écoulement nasal et de la fièvre ont été observées chez les enfants de moins de 15 ans dans le Ndiop. La prévalence de *Streptococcus pneumoniae, Staphylococcus aureus* et *Streptococcus pyogenes* dans la paume des mains a chuté de manière significative dans le Ndiop. L'utilisation du savon a réduit l'incidence des infections respiratoires, des fièvres et la prévalence des bactéries pathogènes sur la peau. Toutefois, pour la diarrhée, des stratégies supplémentaires sont nécessaires pour améliorer les résultats.

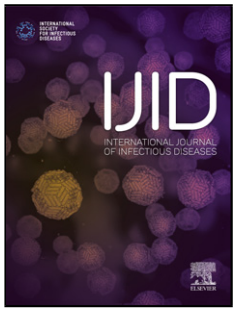
Article IV

# The impact of the daily use of soap in rural areas in Senegal on respiratory infectious diseases, fevers and skin microbiota

Codou Ndiaye, Hubert Bassene, Georges Diatta, Nafissatou Diagne, Parola Philippe, Jean-Christophe Lagier, Cheikh Sokhna and Didier Raoult

Publié dans International journal of intectious diseases

# Journal Pre-proof

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PII: S1201-9712(20)30296-4

DOI: https://doi.org/doi:10.1016/j.ijid.2020.04.076

Reference: IJID 4164

To appear in: *International Journal of Infectious Diseases*

Received Date: 24 January 2020

Revised Date: 25 April 2020

Accepted Date: 28 April 2020

Please cite this article as: Ndiaye C, Bassene H, Diatta G, Diagne N, Philippe P, Lagier J-C, Sokhna C, Raoult D, The impact of the daily use of soap in rural areas in Senegal on respiratory infectious diseases, fevers and skin microbiota, *International Journal of Infectious Diseases* (2020), doi: https://doi.org/10.1016/j.ijid.2020.04.076

# The impact of the daily use of soap in rural areas in Senegal on respiratory infectious diseases, fevers and skin microbiota

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**Text word count: 3457**

**Abstract word count: 200**

## Abstract

**Objectives** Children under five years of age are the group most affected by infectious diseases more specifically in underdeveloped countries. A study was performed to assess the effects of the daily use of soap on the incidence of diarrhea, fever, respiratory infection, and the prevalence of pathogenic bacteria on the skin.

**Methods** Soap was distributed to the population of the village of Ndiop (test) for use in their daily hygiene but not to the population of the village of Dielmo (control). Fieldworkers recorded daily the clinical events in the two villages and encouraged the use of soap in Ndiop.

**Findings** 638 people participated in the study. The incidence rates of cough, runny nose and fevers decreased significantly in 2016 compared with 2015, unlike that of diarrhoea. In 2016, significant reductions in the incidence rates of cough, runny nose and fever were observed in children <15 years old in Ndiop. The prevalence of *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Streptococcus pyogenes* in the palms of hands dropped significantly in Ndiop.

**Conclusion** The use of soap reduces the incidence of respiratory infections, fevers and the prevalence of pathogenic bacteria on the skin. However, for diarrhea, additional strategies are needed to improve outcomes.


**Keywords:** Soap; Hygiene; Infectious diseases; Senegal

## Highlights

- Soap reduces the incidence of respiratory infections and fevers.
- Soap without antibacterial agent does not reduce the incidence of diarrhoea.
- Handwashing reduces the prevalence of pathogenic bacteria.
- Handwashing is a good strategy for controlling infectious diseases.

## Introduction

Diarrhea and pneumonia are the leading causes of death in young children. Of the 6·3 million deaths among children under 5 years of age that occurred in 2013, pneumonia accounted for 15% and diarrhea accounted for 9% [1-3]. Together, these two diseases account for 29% of all deaths in children under 5 years of age, all causes combined, and cause the loss of 2 million young children each year [3]. In Africa, infectious diseases account for 48% of the global under-five deaths [1]. The seriousness of the situation and the lack of coordination in the most affected countries have led the World Health Organization (WHO) and Unicef to set up the integrated Global Action Plan for the Prevention and Control of Pneumonia and Diarrhoea (GAPPD) in 2015 [3]. Handwashing has been proposed as a preventive and inexpensive action for preventing diarrhea and pneumonia [3]. The WHO recommends handwashing with water and soap as the most appropriate measure for reducing diarrhea and pulmonary disease incidences. The incidence of *Shigella* infections in Bangladesh has been significantly reduced as a result of the use of soap for handwashing [4]. A randomized control trial of handwashing performed in Karachi, Pakistan resulted in a 50% reduction in the incidence of pneumonia compared with the control in children under 5 years of age [5] and a reduction of 53% of the incidence of diarrhea in children under 15 years of age [5]. A systematic review concluded that the promotion of handwashing can reduce diarrhea by 47% [6]. Based on current evidence, handwashing with soap can reduce the risk of diarrheal diseases by 42–47% [6]. Approximately half of the world's deaths from pneumonia and diarrhea are recorded in only five countries, most of which are poor and densely populated, namely, India, Nigeria, the Democratic Republic of Congo, Pakistan and Ethiopia [7]. In Senegal, diarrheal diseases are ranked as the third leading cause of death among children under 5 years of age. Their prevalence in 2016 was very high (28%) among children aged 6-23 months [8]. The rate of acute respiratory infections was 3% among children under 5 years of age and 5% among those

aged 12-23 months [8]. In sub-Saharan Africa, socioeconomic and cultural challenges undermine efforts to improve hygiene conditions in the general population [9]. The improvement of health practices in a community is influenced by the knowledge of good hygiene practices, customs and the economic level [10, 11]. Handwashing with soap may not be enough to permanently reduce the incidence of diarrhea and pneumonia in a population. Socioeconomic and cultural factors must be taken into account in the implementation of control strategies [12].

The purpose of this study was to assess the effects of daily soap use in rural areas compared with a control village on the incidence of diarrheal, febrile and respiratory diseases and on the prevalence of pathogenic bacteria on the skin already documented in these villages, such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Rickettsia felis*, *Borrelia crocidurae*, *Tropheryma whipplei*, *Bartonella quintana*, *Streptococcus pyogenes* and *Coxiella burnetii*.

## Materials and methods

### Study area

Dielmo and Ndiop are two Muslim villages in the Fatick region. Dielmo (13°43′N; 16°24′W) is located 280 km southeast of Dakar and 15 km from the northern border of the Republic of The Gambia, both of which belong to the central region of the Sudanese savannah area. Dielmo is located on the banks of a freshwater river, the Néma. The village of Ndiop (13°41′N; 16°22′W) is 5 km from Dielmo and is located in a dry area.. They are populated by farmers and ranchers. The electrification of villages has brought about a profound change in the way of life of the population. Commercial activities are expanding, and the mode of travel has also been revolutionized by the adoption of Jakarta motorbikes [15]. Water consumption from wells is widespread despite the existence of fountains.

Herein, we used Dielmo as the control village and Ndiop as the test village. In the control village, no intervention was undertaken. Preparatory meetings with the inhabitants of the two

villages were organized. The populations were informed and made aware of the objectives of the study and the importance of respecting the instructions. In particular, the inhabitants of the control village were invited to maintain their health and social habits. On the other hand, for the test village, the population was invited to use soap daily for washing hands and body. This study was divided into five parts that were intended to allow us to capture social, clinical, and health events, bacteriological diagnoses and soap use during the study period. Social data and biological samples were obtained through a quarterly survey questionnaire followed by skin swabs taken from the palms of the hands. This activity was carried out by investigators with good levels of experience. Clinical data were collected daily throughout the study by nurses at the health posts in the two villages. The soap supply was the responsibility of a team member. Finally, the isolation of pathogenic bacteria was ensured by a laboratory technician.

**Data collection**

**Participants and procedure**

From January to December 2016, the study was conducted in the villages of Dielmo and Ndiop. The aim was to study the impact of the daily use of an unscented soap (Marseille-type soap) on fevers and respiratory and diarrheal diseases. Data were collected through daily active and passive monitoring in both villages during the period of study. The study design is summarized in Figure 1.

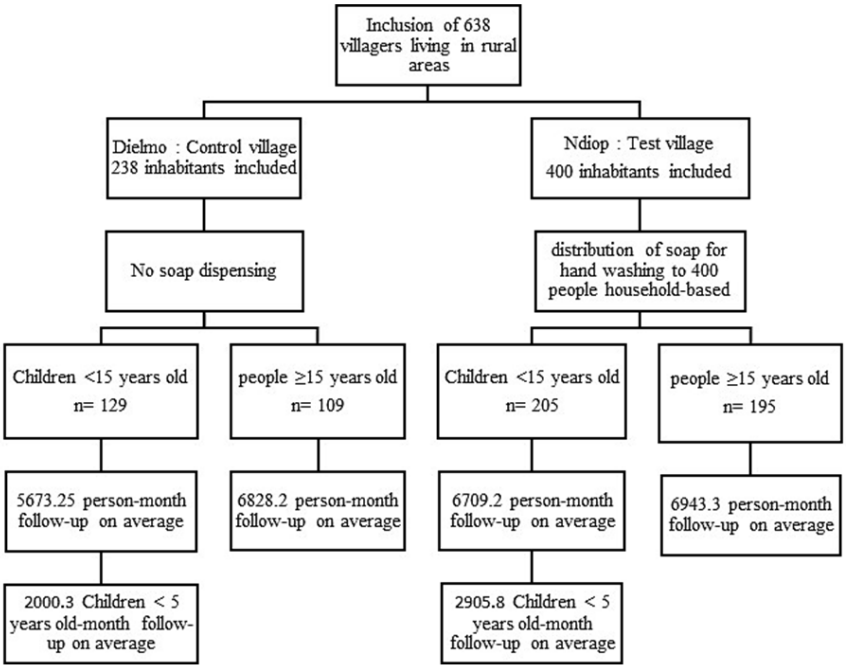


**Figure 1:** The study design

Figure 1 summarizes the different stages of the study, with details of the numbers of children under 5 and 15 years of age.

**Participants**: Residents of all ages and of both sexes from both villages were targeted to participate in the study. In Ndiop, the participants had to commit to complying with the soap use instructions. They also had to agree to use only the soap given to them by the project. In Dielmo, the participants had to commit to not changing their lifestyle or being influenced by the distribution of soap in Ndiop.

**Clinical surveys:** From January to December 2016, the field workers (two per village) met daily with each participant to record their temperatures and check their health. Whenever sought symptoms were detected, the patient was referred to the village health post for a consultation with the nurses. Data on the participant's health status were recorded on the registration documents kept by the investigators, and data from the consultations were recorded on pathological episode forms.

**Soap distribution:** In each village, concessions were divided into households headed by a wife. We used ordinary solid Marseille soap type, based on sodium hydroxide. The soap was

not supplemented with a specific antibacterial agent. During the distribution of soap in the test village, concession chiefs, wives and older children received soap for their personal use, while smaller children were organized into groups of three individuals who shared the same soap. The soaps were first weighed before being given to the participants. After seven days of use, each participant had to return his soap. The soaps were weighed again, and another soap was given to the participant. Thus, the soaps were renewed on a weekly basis. This process of soap delivery and recovery continued throughout the study.

**Swabbing and treatment:** Swabs were performed on the hands after moistening the swab with sterile physiological serum. The swabs were then immerged in an individual tube containing 600 µl 1x phosphate buffered saline (PBS) manufactured by Oxoid Limited (Hampshire, England). Once saturated, the swabs were agitated and pressed against the edges of the tube to release the sample. Then, two 200-µl aliquots of the swab suspension were prepared, one for DNA extraction using the Cetyl Trimethyl Ammonium Bromide (CTAB) 2% method and one for the stock.

**DNA extraction:** Approximately 200µl of the swab bacterial suspension was used for DNA extraction following the CTAB 2% method [16]. At the end of the extraction, the DNA solution was stored for 24 hours in the refrigerator at a temperature of 8°C, as DNA cannot deteriorate after only 24 hours of storage. After qPCR, the DNA was stored at -20°C.

**DNA amplification:** Each reaction was performed at a final volume of 20 µl, containing 10 µl Takyon polymerase, 1 µl of each primer, 1 µl probe, 2 µl RNase-free water and 5 µl DNA. A positive and negative control was included in each run. DNA extracted from the swabbing of a healthy person in Dakar was used as a negative control. The positive control consisted of a suspension made from the swabbing of a healthy person to which bacterial cultures were added. The strains used as positive controls are available on the “Rickettsia Unit Strains Collection (CSUR, WDCM 875)” under the following accession numbers: *S. pneumoniae*

CSURP5700, *S. aureus* CSURP2200 and *S. pyogenes* CSURP6897 [17]. For each species, approximately ten colonies were suspended in 200 µl PBS, and DNA was extracted [17]. The primers and probes used for pathogenic identification are listed below (Table 1). The quality of the DNA handling and the extraction of the swab suspensions was verified by quantitative PCR for a housekeeping gene coding for β-actin, as previously described [17, 18]. We considered samples to be positive if both specific qPCR reactions were positive using a cycle number at the threshold level for a log-based fluorescence (Ct) value lower than 35. This number corresponds to the ability to reveal 10-20 copies of bacterial DNA [18].

**Statistical analysis:** Infectious diseases were assessed by estimating the incidence rate. For each village, the annual incidence rate of a clinical manifestation was calculated as the ratio of the number of registered clinical cases divided by the number of person-times in the year. Monthly incidences were calculated by dividing the number of monthly cases recorded by the number of person-times in the month. The mean incidences of the years 2015 (the year before the intervention) and 2016 (the year of the intervention) were compared using the Fisher exact test to determine the overall impact of soap use on clinical events such as fever, cough, diarrhea and nasal discharge. The differences in incidence rates were analyzed using Epi Info software, version 3.4.1 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Nonparametric values were compared using a $\chi^2$ test. Statistical significance was defined as $p<0.05$.

**Results**

We carried out 372,288 observations, including 174,021 in the control village and 198,267 in the test village. These observations were made on a total of 638 participants, 238 in Dielmo and 400 in Ndiop. Participants aged 0-15 years are in the majority in both villages, with 51·25% (205/400) in the test village and 54·2% (129/238) in the control village (Figure 2). In the two villages, we counted 87 concessions (50 in Dielmo and 37 in Ndiop) and 158

households (72 in Dielmo and 86 in Ndiop). The average number of people per household was 3.3 in the control village and 4·65 in the test village; thus, the number of people per household was significantly higher in the test village ($\chi 2 = 5{\cdot}7$, df=1 $p$=0·02).

We observed 2,707 (1,308 in Ndiop and 1,399 in Dielmo) clinical events in both villages. Events related to respiratory infections were predominant. Households participating in the study received an average of 3 to 4 pieces of soap per week. Each household member used an average of 4 g of soap per day. Incidence rates were highest in the year

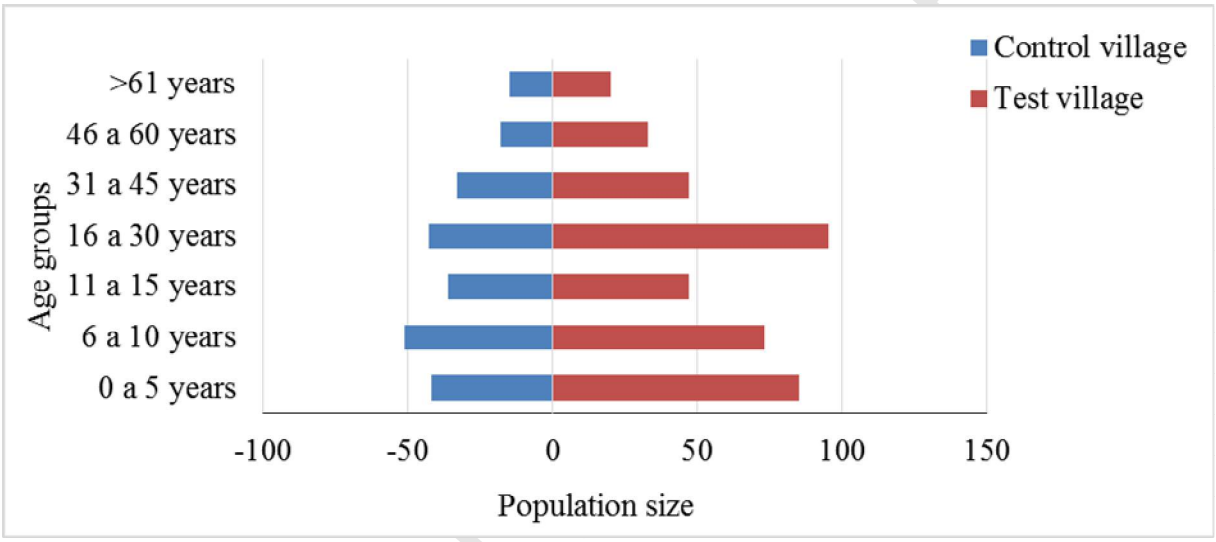


**Figure 2:** Age pyramid of test and control villages

prior to the intervention. Except for diarrhea in the test village, there was a significant decrease in the annual incidence rate of the targeted clinical events in 2016 in both villages (Table 2). Participants < 15 years of age who received soaps presented fewer incidences of coughs, nasal discharges and fevers, and the difference versus the control was significant (Table 3). The difference versus the control was more significant with respiratory problems than with fevers. No significant differences were observed in participants ≥ 15 years in either village. Concerning diarrhea, there was a significant increase in the annual incidence during the period of intervention in Ndiop.

649 swabs were collected during the study. The PCR β-actin was positive for 638 samples, for a prevalence of 98·30% (638/649). The DNA of *B. crocidurae*, *C. burnetii*, *S. pneumoniae*, *S. aureus* and *S. pyogenes* was detected in 273 participants, representing a prevalence of 42·79% (273/638). *S. pneumoniae* was predominant bacteria, with a prevalence of 31·81 (203/638), followed by *S. aureus* at 25·39% (162/638) and *S. pyogenes* at 1·88% (12/638). The prevalences of bacteria before intervention ($T_0$) were higher than those obtained during the intervention (Table 3). Before the intervention, the difference in the prevalence of *S. aureus* between the villages test and control was not significant. However, the differences concerning *S. aureus* and *S. pneumoniae* were significant during the period of intervention (Table 3). *S. pyogenes* was absent in Ndiop before the distribution of soap. During the first two quarters of the intervention, this bacteria was completely absent in the two villages; however, during the last quarter, we noted a resurgence of *S. pyogenes* in both villages, with a significantly higher prevalence in Dielmo (Table 4). During the period before the intervention, the prevalence of *S. aureus* on the palms of the hands was significantly higher in Dielmo ($\chi2 = 9·24$, df=1, $p$=0·002) and that of *S. pneumoniae* was also significant ($\chi2 = 40·02$, df=1, $p$=1 x 10-7); however, the difference between the prevalence of *S. pyogenes* in the test and control villages could not be verified because one of the values was lower than 5 (Figure 3). The prevalence of *S. aureus* in children under 15 years of age before the soap distribution ($T_0$) was significantly higher than the average prevalence during the coverage period ($\chi2 = 4·51$, df=1 $p$=0·04); this outcome was also found in *S. pneumoniae* ($\chi2 = 45·91$, df=1, $p$=1 x 10-7).

Before the intervention, the prevalence of *S. aureus* on the palms of the hands was significantly higher in Dielmo ($\chi2 = 9·24$, df=1, $p$=0·002) and that of *S. pneumoniae* was also significant ($\chi2 = 40·02$, df=1, $p$=1 x 10-7). *S. pyogenes* was absent Ndiop before the distribution of soap (Figure 4). The prevalence of *S. aureus* in children under 5 years of age before the soap distribution (T0) was significantly higher than the average prevalence during

the coverage period ($\chi 2 = 4{\cdot}51$, df=1, $p$=0·04); this result was also found for *S. pneumoniae* ($\chi 2 = 45{\cdot}91$, df=1, $p$=1 x 10-7). Among participants over 15 years of age, the prevalence of different pathogens before soap distribution was significantly higher in Dielmo. Values were obtained for *S. aureus* ($\chi 2 = 14{\cdot}96$, df=1, $p$=1 10-4) and *S. pneumoniae* ($\chi 2 = 9{\cdot}997$, df=1, $p$=1 10-3). However, for *S. pyogenes*, the significance could not be measured because no

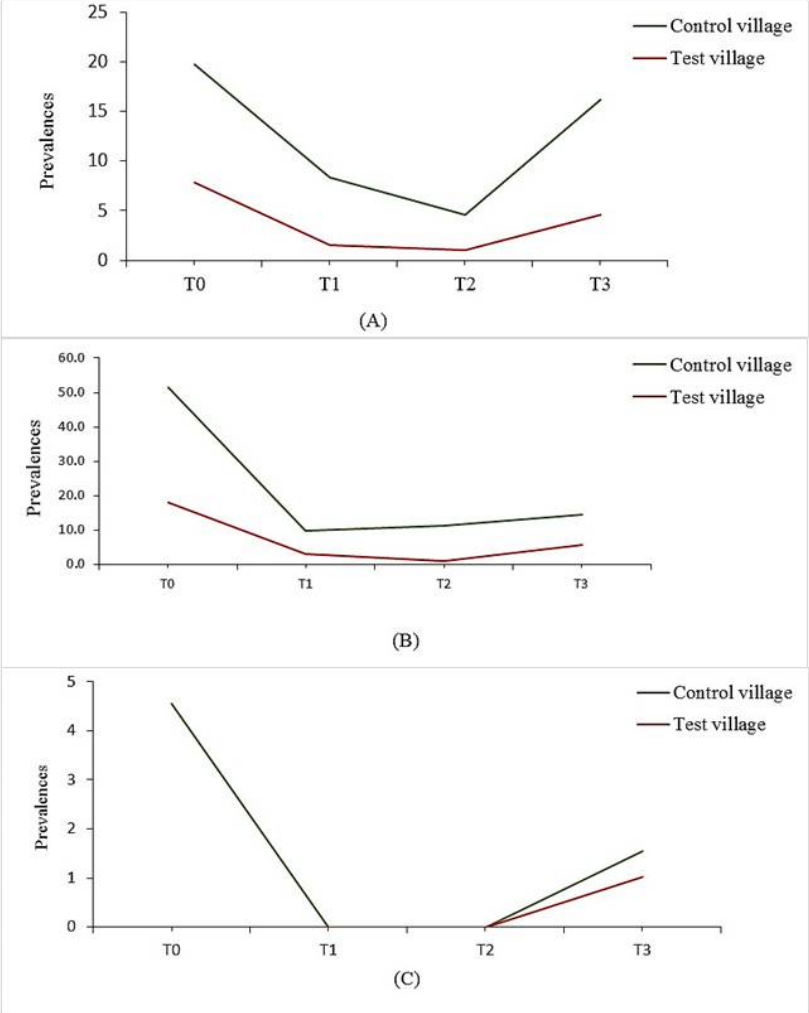


**Figure 3**: Evolution of bacterial prevalence on the palm of the hands of children <15 years of age. (A) Evolution of the prevalence of *S. aureus* in children <15 years of age. $T_0$ refers to before the distribution of soap, and $T_1$ to $T_3$ refer to periods during the soap use. (B) Evolution of the prevalence of *S. pneumoniae* and (C) that of *S. pyogenes*.

contamination was detected in Ndiop during this period (Table 5). The number of people infected with *S. aureus* during the intervention in Ndiop decreased significantly during the

period of soap use. The number of people infected with *S. pneumoniae* also decreased, but the *p* value could not be measured because the total contamination was less than 5. However, for *S. pyogenes,* no contamination was detected before or after the distribution of soap in Ndiop (Table 5).

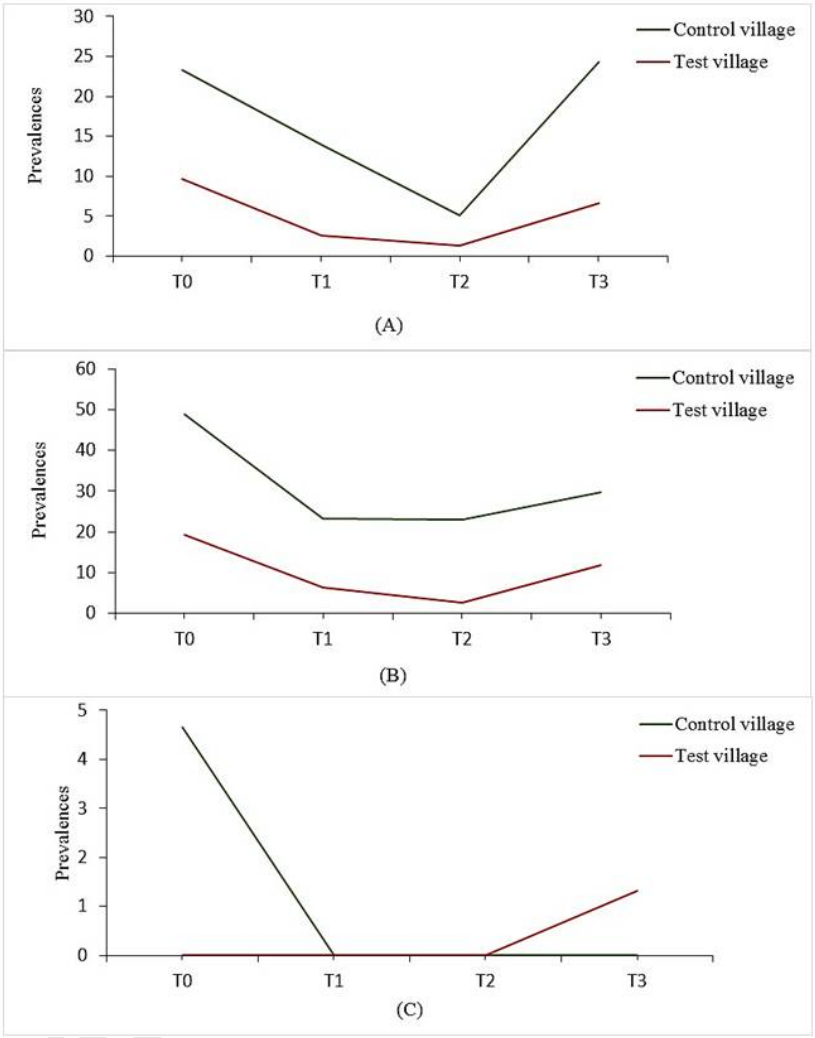


**Figure 4:** Evolution of bacterial prevalence on the palm of the hands of children <5 years of age. (A) Evolution of the prevalence of *S. aureus* in children <5 years of age. $T_0$ refers to before the distribution of soap, and $T_1$ to $T_3$ refer to periods during the soap use. (B) Evolution of the prevalence of *S. pneumoniae* and (C) that of *S. pyogenes.*

**Bibliographic research**

Most control strategies aimed at eliminating skin pathogens have targeted those pathogens responsible for diarrhea in children or health persons. Swabs were taken either from the palms of the hands or from specific parts of the body (Table 6). Previous studies that have assessed the impact of soap on the occurrence of respiratory infections, fevers and diarrhea have yielded mixed results. While the majority have agreed that handwashing with soap and water helps to reduce the incidence of these diseases, some studies have not been as successful (Table 7).

**Discussion**

.This work, which was carried out in rural Senegal, presents the beneficial effects of soap use on the incidence of respiratory diseases and fevers. The population generally adhered well to the project with a very high participation rate. The soap was correctly used by the participants for their hygiene.

In both villages, the incidence of fevers and respiratory diseases decreased significantly between 2015 and 2016. However, the number of people with diarrhea did not decrease significantly. Diarrhea is a disease with multiple causes (bacterial, parasitic, functional, etc.), and the use of soap may not be sufficient to reduce its incidence rate. The pathogens are usually transmitted through the fecal-oral route [19] by contaminated drinking water or the consumption of contaminated food.

The use of soap in households was not controlled, and the participants were aware of the benefits and importance of soap in the fight against infectious diseases. However, unlike previous studies that have focused on the promotion of handwashing to fight nosocomial infectious diseases [20-22] in poor neighborhoods [5], the use of soap in this study was not supervised. Despite this lack of control, the annual incidence rate of respiratory diseases and fevers decreased significantly among the participants under 15 years of age residing in the test village. These results show that the participants took ownership of the study objectives and

that soap use was beneficial for this segment of the population. The adoption of basic hygiene rules such as hand and body washing with soap has had a positive impact on the occurrence of fevers and respiratory infections [5, 23, 24] (Table 6). It also has had a negative impact on the development and multiplication of pathogenic bacteria living on the skin [25-30] (Table 5). Studies have shown that in some areas, ethnic, religious and social factors must be taken into account to ensure the success of such a strategy [31]. Our results suggest that it is mainly adults who are the problem. In this segment of the population, no significant differences were recorded for all targeted diseases. The largest decreases were recorded among children under 5 years of age. This is a segment of the population that is generally under the care of their mothers. This could explain the approximate 2-point decrease in incidence the rate. During the year of intervention, no significant differences were recorded in the occurrence of diarrhea.

The qPCRs performed on the swabs show that bacteria that may have caused the clinical manifestations recorded during the study period were present on the palms of the hands. Other bacteria, such as *S. pneumoniae*, *S. pyogenes* and *S. aureus*, are involved in respiratory and skin infections specifically *S. pyogenes* and *S. aureus*. This is why their presence in the palms of the hands is an important factor in the spread of respiratory infections in this area. The prevalence of *S. pneumoniae*, *S. pyogenes* and *S. aureus* was much lower in Ndiop. This result is probably due to the use of soap in the personal hygiene routines of the Ndiop population. We suspected that the population was slackening in terms of their compliance with the study rules outlined at the beginning of the study in the third trimester. This situation could explain the presence of *S. pyogenes* in children under 5 years of age in the third trimester after the soap distribution began. Among the participants over 15 years of age, we also observed a decrease in the prevalence of bacteria. Previous studies [32, 33] have shown that the palms of the hands are home to many pathogenic bacteria that could spread through skin contact. *S.*

306 *pneumoniae*, *S. pyogenes* and *S. aureus* are involved in respiratory tract infections; in
307 children, 15% of these respiratory tract infections are pneumonia [34].
308 Some limitations could be noted in this study, notably the impossibility of measuring the real
309 effect of soap on the reduction of targeted pathologies. It would have been necessary to
310 deprive the population of the village control of soap for their daily hygiene (which would be
311 ethically unacceptable). The soap we used was based on sodium hydroxide, it was not
312 supplemented with an antibacterial agent.
313 The identification of these bacteria on the palms of the participants and the significant
314 reduction in their presence after soap use proves that promoting personal hygiene through
315 simple and inexpensive actions such as handwashing and body washing with soap can
316 substantially reduce the incidence of infectious diseases.

317 **Conclusion**

318 This study shows that a simple and inexpensive strategy based on hand and body washing
319 with soap and water could significantly reduce the incidence of respiratory infections, febrile
320 diseases and pathogenic bacteria development. The efficacy of handwashing with soap on
321 diarrhea was not demonstrated in this study. Additional measures, such as good public
322 awareness, care for malnourished children, increased drinking water supplies, etc., must be
323 combined with handwashing for the reduction of incidence rates. Based on these results, it
324 appears necessary to undertake a study evaluating the sustainability of behavioral change in
325 handwashing promotion, and it is also important to assess cost-effectiveness. Finally, the
326 country's health authorities should take these results into account and promote handwashing
327 with soap and water in their infectious disease prevention programs.

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330 **Conflict of interest**

No conflict of interest was declared by the authors.

**Acknowledgements**

We would like to thank the Mediterranean Infection Foundation and the french National Research Agency through the "Investissement D'avenir program" (Grant Number ANR-10-IAHU-03) which funded this study. We also would like to thank the technical staff of the Dielmo and Ndiop project for their contribution to the data and sample collection, and the people of both villages for their participation and collaboration in this study.

## Ethical Approval

This study was authorized by the National Ethics Committee for Health Research (CNERS), No. 00053/Ministry of Health and Social Action (MSAS)/ Direction of Planning, Research and Statistics (DPRS)/CNERS.

## Author Contribution

**Data collection:** Hubert Bassene, Codou Ndiaye, Nafissatou Diagne

**Writing the first draft:** Codou Ndiaye, Hubert Bassene, Cheikh Sokhna, Jean-Christophe Lagier, Didier Raoult

**Satistical analyses:** Hubert Bassene

**Paper review:** Didier Raoult, Cheikh Sokhna, Jean-Christophe Lagier

**Approbation of the final version:** All authors

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450 **Table 1: Target sequences, primers and probes used in the study**

| Targeted Organism | Targeted gene | Name | Sequences | References |
|---|---|---|---|---|
| **All *Rickettsia* except *R. typhi* and *R. prowazekii*** | RC0338 | 1029-F1 | GAM AAA TGA ATT ATA TAC GCC GCA AA | [17] |
| | | 1029-R1 | ATT ATT KCC AAA TAT TCG TCC TGT AC | |
| | | Rick1029_MBP | 6FAM- CGG CAG GTA AGK ATG CTA CTC AAG ATA A-TAMRA | |
| ***R. felis*** | Biotin synthase | R_fel0527_F | ATG TTC GGG CTT CCG GTA TG | [17] |
| | | R_fel0527_R | CCG ATT CAG CAG GTT CTT CAA | |
| | | R_fel0527_P | 6FAM- GCT GCG GCG GTA TTT TAG GAA TGG G -TAMRA | |
| **All *Bartonella*** | ITS | Barto_ ITS3_ F | GAT GCC GGG GAA GGT TTT C | [17] |
| | | Barto_ ITS3_ R | GCC TGG GAG GAC TTG AA CCT | |
| | | Barto _ITS3 _P | 6FAM- GCG CGC GCT TGA TAA GCG TG -TAMRA | |
| **All *Borrelia*** | 16S | Bor_16S_3_F | AGC CTT TAA AGC TTC GCT TGT AG | [17] |
| | | Bor_16S_3_R | GCC TCC CGT AGG AGT CTG G | |
| | | Bor_16S_3_P | 6FAM-CCG GCC TGA GAG GGT GAA CGG-TAMRA | |
| ***B. crocidurae*** | glpQ | Borcro_glpQ_F | CCT TGG ATA CCC CAA ATC ATC | [17] |
| | | Borcro_glpQ_R | GGC AAT GCA TCA ATT CTA AAC | |
| | | Borcro_glpQ_MGB_P | 6FAM-ATG GAC AAA TGA CAG GTC TTAC-MGB | |
| ***C. burnetii*** | IS1111A | Coxbur_IS1111_0706_F | CAA GAA ACG TAT CGC TGT GGC | [17] |
| | | Coxbur_IS1111_0706_R | CAC AGA GCC ACC GTA TGA ATC | |
| | | Coxbur_IS1111_0706_P | 6FAM-CCG AGT TCG AAA CAA TGA GGG CTG-TAMRA | |
| | Hyp. Protein | Coxbur_IS30A_3_F | CGC TGA CCT ACA GAA ATA TGT CC | [17] |
| | | Coxbur_IS30A_3_R | GGG GTA AGT AAA TAA TAC CTT CTG G | |
| | | Coxbur_IS30A_3_P | 6-FAM- CAT GAA GCG ATT TAT CAA TAC GTG TAT GC-TAMRA | |
| ***S. aureus*** | NucA | Saur__NucA__F | TTG ATA CGC CAG AAA CGG TG | [17] |
| | | Saur__NucA__R | TGA TGC TTC TTT GCC AAA TGG | |
| | | Saur__NucA_MGB_P | 6FAM- AAC CGA ATA CGC CTG TAC -MGB | |
| | *Amidohydrolase* | Saur_F | CCT CGA CAG GTA ACG CAT CA | [17] |
| | | Saur_R | AAA CTC CTA TCG GCC GCA AT | |
| | | Saur_P | 6FAM-TGC AAT GGT AGG TCC TGT GCC CA | |

| | | | | |
|---|---|---|---|---|
| ***S. pyogenes*** | hypothetical | Spyo_hypp_F | ACA GGA ACT AAT ACT GAT TGG AAA GG | [17] |
| | | Spyo_hypp_R | TGT AAA GTG AAA ATA GCA GCT CTA GCA | |
| | | Spyo_hypp_P | 6FAM- AAAATGTTGTGTTTTAGGCACTGGCGG-TAMRA | |
| | MipB | Spyo_mipB_F | CCA TAC GGT TAT AGT AAG GAG CCA AA | [17] |
| | | Spyo_mipB_R | GGC TAT CAC ATC ACA GCA ACC | |
| | | Spyo_mipB_P | 6FAM- TCAGCGCCAGCTTCAATGGC- TAMRA | |
| ***S. pneumoniae*** | plyN | Pneumo_plyN_F | GCG ATA GCT TTC TCC AAG TGG | [17] |
| | | Pneumo_plyN_R | TTA GCC AAC AAA TCG TTT ACC G | |
| | | Pneumo_plyN_P | 6FAM-CCC AGC AAT TCA AGT GTT CGC CGA-TAMRA | |
| | lyt A | Pneumo_lytA_F | CCT GTA GCC ATT TCG CCT GA | [17] |
| | | Pneumo_lytA_R | GAC CGC TGG AGG AAG CAC A | |
| | | Pneumo_lytA_P | 6-FAM- AGA CGG CAA CTG GTA CTG GTT CGA CAA-TAMRA | |
| ***T. whipplei*** | WiSP family protein (WHI2) | T_whi2_F | TGA GGA TGT ATC TGT GTA TGG GAC A | [17] |
| | | T_whi2_R | TCC TGT TAC AAG CAG TAC AAA ACA AA | |
| | | T_whi2_P | 6FAM- GAG AGA TGG GGT GCA GGA CAG GG-TAMRA | |
| | WiSP family protein (WHI3) | T_whi3_F | TTG TGT ATT TGG TAT TAG ATG AAA CAG | [17] |
| | | T_whi3_R | CCC TAC AAT ATG AAA CAG CCT TTG | |
| | | T_whi3_P | 6FAM- GGG ATA GAG CAG GAG GTG TCT GTC TGG-TAMRA | |

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**Table 2:** Comparison of annual incidence rates for the years 2015 and 2016

| | Cough | | | Nasal discharge | | | Diarrhea (≥4 stool/day) | | | | Fever | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Years** | **Annual incidence rate 2015** | ***p* (95% CI) 2016** | **Annual incidence rate** | ***p* (95% CI) 2015** | **Annual incidence rate 2016** | ***p* (95% CI)** | **Annual incidence rate 2015** | ***p* (95% CI) 2016** | **Annual incidence rate 2015** | ***p* (95% CI) 2016** | **Annual incidence rate** | ***p* (95% CI) 2015** |
| **Dielmo** | 14·84 | 12·51 | $P = 0{\cdot}005$ | 12·41 | 10.1 | $P = 0{\cdot}005$ | 3·4 | 1·7 | 14·84 | 12·51 | $P = 0{\cdot}005$ | 12·41 |
| **Ndiop** | 12·72 | 9·59 | $P = 1\ 10^{-5}$ | 10·12 | 8·16 | $P = 0{\cdot}003$ | 4·31 | 3·68 | $P = 0{\cdot}16$ | 10·4 | 7·0 | $P = 1\ 10^{-7}$ |

**Table 3:** Comparison of incidence rates

| | Participant < 5 years | | | Participant < 15 years | | | Participant ≥15 years | | |
|---|---|---|---|---|---|---|---|---|---|
| | Annual incidence rate | | *p* (95% CI) | Annual incidence rate | | *p* (95% CI) | Annual incidence rate | | *p* (95% CI) |
| | Test (n=31,964) | Control (n=24,004) | | Test (n=73,801) | Control (n=68,079) | | Test (n=76,376) | Control (n=81,938) | |
| **Cough** | 1·08 | 2·52 | $P = 1\ 10^{-7}$ | 1·86 | 3·22 | $P = 1\ 10^{-7}$ | 0·94 | 0·82 | $P = 0{\cdot}18$ |
| **Nasal discharge** | 1·12 | 3·16 | $P = 1\ 10^{-7}$ | 0·89 | 1·45 | $P = 2\ 10^{-5}$ | 0·72 | 0·63 | $P = 0{\cdot}26$ |
| **Diarrhea (≥4 stool/day)** | 0·87 | 0·87 | $P = > 0.99$ | 0·11 | 0·09 | $P = 0.41$ | 0·41 | 0·25 | $P = 0{\cdot}004$ |
| **Fever** | 1·16 | 1·90 | $P = 3\ 10^{-4}$ | 0·91 | 1·31 | $P = 0.004$ | 0·46 | 0·40 | $P = 0{\cdot}36$ |

**n:** number of observations in the year

**Table 4:** Comparison of the prevalence of bacteria between villages test and control before and after the intervention

| | Bacteria | Control | Test | *p* |
|---|---|---|---|---|
| **$T_0$** | *S. pneumoniae* | 49% (116/238) | 21·75% (87/400) | $P = 1\ 10^{-7}$ |
| | *S. pyogenes* | 5% (12/238) | 0% | |
| | *S. aureus* | 30% (71/238) | 22·75% (91/400) | $P = 0{\cdot}05829$ |
| **$T_{01}$** | *S. pneumoniae* | 5·5% (13/238) | 1·75% (7/400) | $P = 0{\cdot}01792$ |
| | *S. pyogenes* | 0% | 0% | |
| | *S. aureus* | 6·30% (15/238) | 0·75% (3/400) | $P = 0{\cdot}0001186$ |
| **$T_{02}$** | *S. pneumoniae* | 7·56% (18/238) | 1·0% (4/400) | $P = 0{\cdot}00003054$ |
| | *S. pyogenes* | 0% | 0% | |
| | *S. aureus* | 3·0% (7/238) | 0·75% (3/400) | $P = 0{\cdot}07$ |
| **$T_{03}$** | *S. pneumoniae* | 10·0% (23/238) | 3·0% (12/400) | $P = 0{\cdot}0006860$ |
| | *S. pyogenes* | 3·0% (7/238) | 0·5% (2/400) | $P = 0{\cdot}02915$ |
| | *S. aureus* | 10·0% (23/238) | 3·0% (11/400) | $P = 0{\cdot}0006$ |

**Table 5:** Prevalence of bacteria on the palms of the hands in participants ≥15 years

| | $T_0$ | | $T_1$ | | $T_2$ | | $T_3$ | |
|---|---|---|---|---|---|---|---|---|
| | **Control** | **Test** | **Control** | **Test** | **Control** | **Test** | **Control** | **Test** |
| ***S. aureus*** | 31% (34/111) | 12% (24/199) | 4% (4/111) | 0 | 1% (1/111) | 0·5% (1/205) | 2% (2/113) | 1% (2/206) |
| ***S. pneumoniae*** | 43% (48/111) | 25% (50/199) | 0 | 0·5% (1/202) | 3% (3/111) | 1% (2/205) | 3% (4/113) | 0·5% (1/206) |
| ***S. pyogenes*** | 5% (6/111) | 0 | 0 | 0 | 0 | 0 | 4% (5/113) | 0 |

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476 **Table 6**: Effect of interventions on pathogenic bacteria living on the human skin

| Study | Study population | Intervention component | Pathogens |
|---|---|---|---|
| Chen et *al.* (2015) | Neonates (preterm) Born <33 weeks gestational age (Mean age = 2 days) | Hand hygiene education | Invasive Candida infections |
| Raza et *al.* (2013) | Neonates (ages not reported) | Clean care delivery kits (soap, spirit for clean cord care, sterile equipment) | Neonatal tetanus infection |
| Garza et *al.* (2017) | Ages not reported | Hand decontaminated | *Escherichia coli* and *Enterococcus faecalis* |
| Jensen et *al.* (2017) | 24·5 ± 3·9 years (mean ± SD) | Hand washing | *Escherichia coli* ATCC 11229 |
| de Aceituno et *al.* (2015) | Ages not reported | Hands washing | *Escherichia coli*, and *Enterococcus* spp |
| Yu et *al.* (2018) | 18 to 75 years | Four skin sites (right ankle, right medial forearm, right outer hand, right armpit) | Skin microbiota |
| Barker et *al.* (2017) | ≥ 8 years | Hand hygiene | *Clostridium difficile* |

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480 **Table 7**: Effect of interventions on the occurrence of pathological episodes

| Study | Study population | Estimator | Diagnosed diseases | Difference vs control effect estimate (95% CI) | Reduction observed |
|---|---|---|---|---|---|
| Larson et *al.* (2004) | Children <5 years | Risk | Cough | 0·97 (0·79–1.18) | No statistically significant differences between intervention and control |
| | | | Nasal discharge | 1·03 (0·81–1·32) | |
| | | | Diarrhea | 0·90 (0·54–1·50) | |
| | | | Fever | 0·84 (0·63–1·12) | |
| Luby et *al.* (2005) | Children <15 years | Mean incidence | Cough | -50% (-65% to -36%) | Statistically significant differences between intervention and control were observed |
| | | | Diarrhea | -50% (-64% to -37%) | |
| Rabie et *al.* (2006) | Ages not reported | Relative risk | Acute respiratory infection | 16% (11%–21%) | Statistically significant differences between intervention and control were observed |
| Kim et *al.* (2018) | Adults | Incidence rate | Acute respiratory illness | 13·0% (10·6%–15·9%) | Statistically significant differences between intervention and control were observed |
| Pickering et *al.* (2019) | 18–24 months | Prevalence | Diarrhea | 27% in the control group SHINE trials (10% in the control group) | Reduction was observed in Bangladesh but not in Kenya or Zimbabwe |

| | | | | | |
|---|---|---|---|---|---|
| Slayton et *al.* (2016) | < 2 years | Rates per 100 person-visits | Diarrhea | - | No statistically significant differences between intervention and control |
| Humphrey et *al.* (2019) | 18 month | Prevalence ratio | Diarrhea | 1·18 (0·87–1·61) | No statistically significant differences between intervention and control |
| | | | Acute respiratory infection | 1·75 (0·62–4·90) | |
| Chard et *al.* (2019) | Pupils in schools | Risk | Diarrhea | 0·80 (0·51–1·26) | Reduction was not observed |
| | | | Respiratory infections | 1·08 (0·95–1·23) | |

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# Partie V

Description des nouvelles espèces isolées au cours de ce travail par taxonogenomics.

Avant propos

L'exploration du microbiote cutané par culturomics a permis l'isolation de 6 nouvelles espèces. Dans ce chapitre, nous présentons les descriptions de ces nouvelles espèces isolées. Parallèlement aux avancées technologiques, la description de nouvelles espèces bactériennes a subi de nombreuses modifications au cours du temps. Initialement basées sur des critères phénotypiques (Lehmann *et al*.,1920), la classification des bactéries a été révolutionnée par la détermination du contenu en G+C (De Ley *et al*.,1974; Sneath *et al*.,1973.) ainsi que l'hybridation ADN-ADN considéré comme « gold standard » de la taxonomie bien que n'étant pas reproductible et est réservée seulement à quelques laboratoires (Tindall *et al.*,2010). Actuellement, la taxonomie bactérienne repose donc sur une méthode innovante introduite par notre équipe, la taxonogénomique. La taxonogénomique intègre à la fois l'information phénotypique (correspondant aux critères morphologiques, physiologiques, chimiotaxonomiques, ainsi que les caractéristiques culturales) Chacune des nouvelles espèces découvertes par Culturomics est décrite selon la méthode de NSA2.0. Dans cette nouvelle formule, en plus des caractéristiques phénotypiques une comparaison du génome avec les espèces les plus proches y est ajoutée. Il s'agit de l'identité nucléotidique moyenne des gènes orthologues, calculé par le logiciel en ligne OrthoANI (https://www.ezbiocloud.net/tools/orthoani ). Une espèce bactérienne est considérée comme nouvelle lorsque sa séquence de l'ARN ribosomale 16S a un pourcentage de similarité inférieur ou égale à 98,7% par rapport aux espèces, phylogénétiquement, les plus proches comme précédemment décrit (Stackebrant, 2011). Dans le but de communiquer plus rapidement le grand nombre de bactéries identifiées, à la communauté scientifique, nous avons parallèlement mis en place un format intermédiaire nommé "new species announcement" (Drancourt *et al*., 2018; Fournier *et al*., 2017). Il comporte la description phénotypique et les informations phylogénétiques de l'espèce. Dans cette partie, nous présenterons la description en format NSA2.0 de 5 espèces nouvelles bactéries isolées et 1 nouvelle espèce de bactérie annoncée sous le format de "new species announcement".

Article 5

# *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., and *Erwinia mediterraneensis* sp. nov., three new species isolated from the human skin

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Publié dans New Microbes and New Infections

# *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., and *Erwinia mediterraneensis* sp. nov., three new species isolated from the human skin

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## Abstract

*Lysinibacillus timonensis* strain Marseille-P5727$^T$ (=CSURP5727), *Microbacterium timonense* strain Marseille-P5731$^T$ (=CSURP5731) and *Erwinia mediterraneensis* strain Marseille-P5165$^T$ (=CSURP5165) are three new species isolated from the human skin.


**Keywords:** Culturomics, *Erwinia mediterraneensis*sp. nov., Human skin, *Lysinibacillus timonensis* sp. nov., *Microbacterium timonens* esp. nov.
**Original Submission:** 27 May 2019; **Accepted:** 12 June 2019
**Article published online:** 20 June 2019

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## Introduction

The skin is the biggest protective organ in humans. However, the cutaneous microbiota is involved in many skin diseases and plays a role in wound infections [1]. The majority of bacteria colonizing human skin belong to three phyla: *Firmicutes*, *Actinobacteria* and *Proteobacteria* [2].

As part of a culturomics study of the human microbiota [3,4], we isolated from skin samples of three different healthy Senegalese individuals, three new bacteria that could not be identified by our systematic matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany). Each of the three species has been described according to their following main phenotypic description, phylogenetic analysis and genome sequencing [5,6].

### Isolation and growth conditions

In 2017, we isolated three unidentified bacterial strains from the skin swabs of an 18-year-old man, a 35-year-old man living in Ndiop and a 15-year-old girl living in Dielmo. The study was validated by the ethics committee of Senegal (No. 53/MSAS/DPRS/CNERS on 31 March 2015). A screening was made by MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics) as previously described [7]. The spectra obtained (Fig. 1) were imported into MALDI BIOTYPER 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database constantly updated with MEPHI database). The initial growth of *Lysinibacillus timonensis* sp. nov., strain Marseille-P5727, was obtained after 24 h of culture on a Chapman–mannitol salt agar (BioMérieux, Marcy l'Etoile, France) in aerobic conditions at 37°C and pH 7.4. *Microbacterium timonense* sp. nov., strain Marseille-P5731 and *Erwinia mediterraneensis* sp. nov., strain Marseille-P5165 both grew on Columbia Colistin and Nalidixic Acid agar supplemented with 5% sheep blood (BioMérieux) under aerobic conditions at 37°C with pH 7.3.

### Strain identification

The 16S rRNA gene was sequenced to classify these bacteria. Amplified by using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequencing using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer



https://doi.org/10.1016/j.nmni.2019.100579

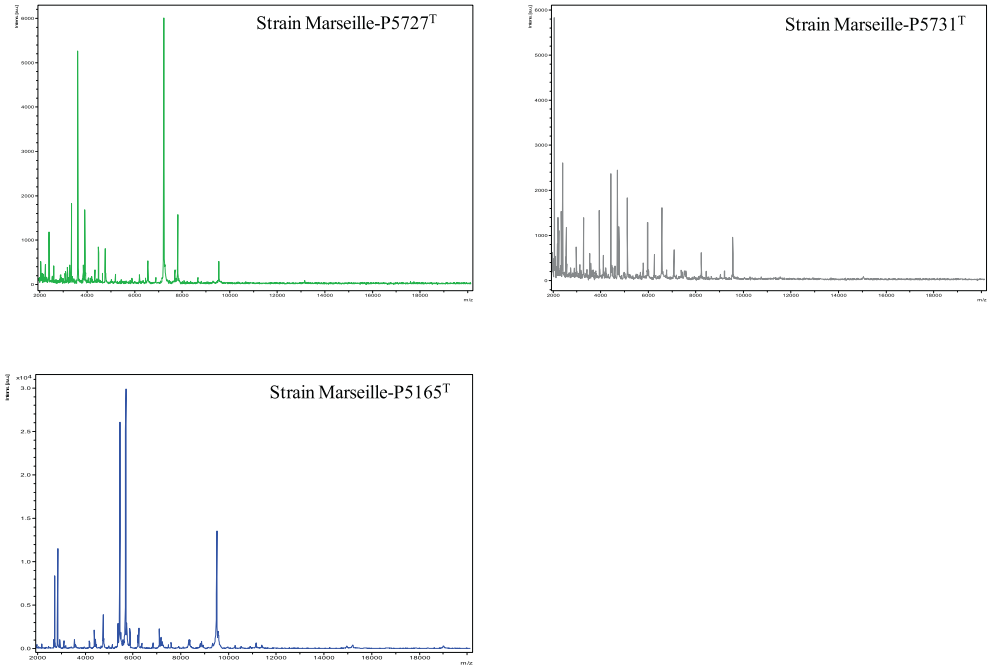


**FIG. 1.** MALDI-TOF MS reference spectrum of the three new species described above. The reference spectra were generated by comparison of spectra from 12 individual colonies for each species.

capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [8]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P5727$^T$ exhibited a 95.47% sequence identity with *Lysinibacillus endophyticus* strain C9 (GenBank Accession number NR_146821.1), the phylogenetically closest species with standing in nomenclature. Strain Marseille-P5731$^T$ exhibited a 98.08% sequence identity with *Microbacterium hominis* strain DSM 12509 (GenBank Accession number: NR_042480.1), the phylogenetically closest species with standing in nomenclature. Strain Marseille-P5165$^T$ exhibited a 97.46% sequence identity with *Erwinia iniecta* strain B120 (GenBank Accession number NR_137333.1), the phylogenetically closest species with standing in nomenclature. Based on the 16S rRNA sequences, we consequently rank the strains as members of new species within the following three phyla: *Proteobacteria*, *Actinobacteria* and *Firmicutes* (Fig. 2).

## Phenotypic characteristics

*Lysinibacillus timonensis* strain Marseille-P5727$^T$ presents opaque colonies with a greyish colour. Bacterial cells were Gram-negative, non-motile, spore-forming, club-shaped rods, 3.74 µm long and 0.61 µm wide. Strain Marseille-P5727$^T$ showed catalase-positive and oxidase-negative activities. *Microbacterium timonense* strain Marseille-P5731$^T$ has grey colonies with irregular edges, creamy. Bacterial cells were Gram-negative, motile and measured 1.25 µm in length and 0.53 µm in width. Strain Marseille-P5731$^T$ showed catalase activity, but not oxidase activity. *Erwinia mediterraneensis* strain Marseille-P5165$^T$ presented colonies with a smooth surface and regular edges. Cells stained as Gram-negative. They are motile and measure means of 1.98 µm long and 0.62 µm wide. Strain Marseille-P5165$^T$ is catalase-positive and oxidase-positive (Fig. 3).

## Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA Tissue Kit and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired end (Illumina), as previously described [9]. Assembly was performed with a pipeline incorporating different softwares (Velvet [10], Spades [11] and Soap Denovo [12]), and trimmed data (MiSeq and Trimmomatic [13] softwares) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value <25% of the mean depth were removed. The best assembly was selected using different criteria (number of scaffolds, N50, number of N). The degree of genomic similarity of these three strains with closely related species was estimated using OrthoANI software [14]. The genome of strain Marseille-P5727$^T$ was 4 220 229 bp long with a 35.7 mol% G+C content. OrthoANI values among closely related species ranged from 70.55% between *Lysinibacillus manganicus* and *Lysinibacillus*

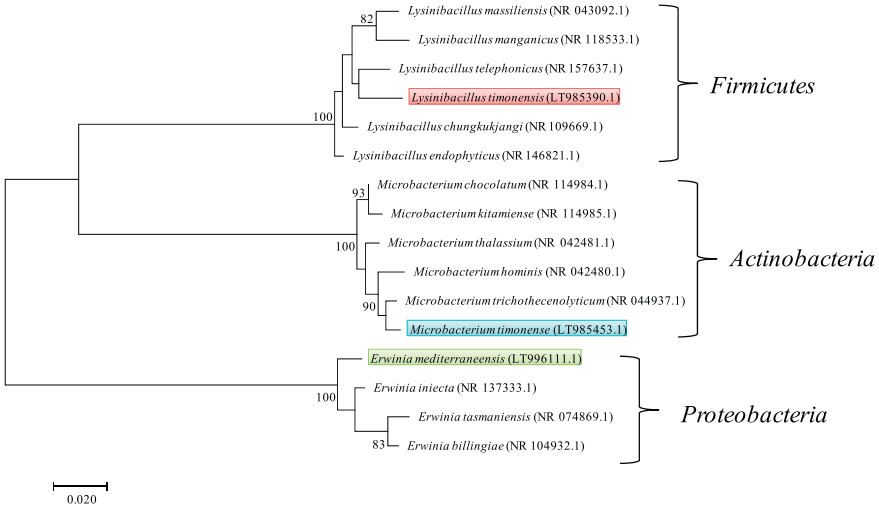


**FIG. 2.** Phylogenetic tree showing the position of *Lysinibacillus timonensis* strain Marseille-CSURP5727$^T$, *Microbacterium timonense* strain Marseille-CSURP5731$^T$ and *Erwinia mediterraneensis* strain Marseille-CSURP5165$^T$ relative to other phylogenetically closest species. The respective GenBank Accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 2% nucleotide sequence divergence.

*odysseyi* to 84.36% between *Lysinibacillus boronitolerans* and *Lysinibacillus fusiformis*. When *Lysinibacillus timonensis* was compared with these closely related species, values ranged from 71.21% with *Microbacterium boronitolerans* to 77.78% with *Lysinibacillus massiliensis*.

The genome of strain Marseille-P5731$^T$ was 3 961 226 bp long with a 70.1 mol% G+C content. ORTHOANI values among closely related species ranged from 65.81% between *Microbacterium hominis* and *Microbacterium oleivorans* to 83.27% between *Microbacterium timonense* and *Microbacterium*

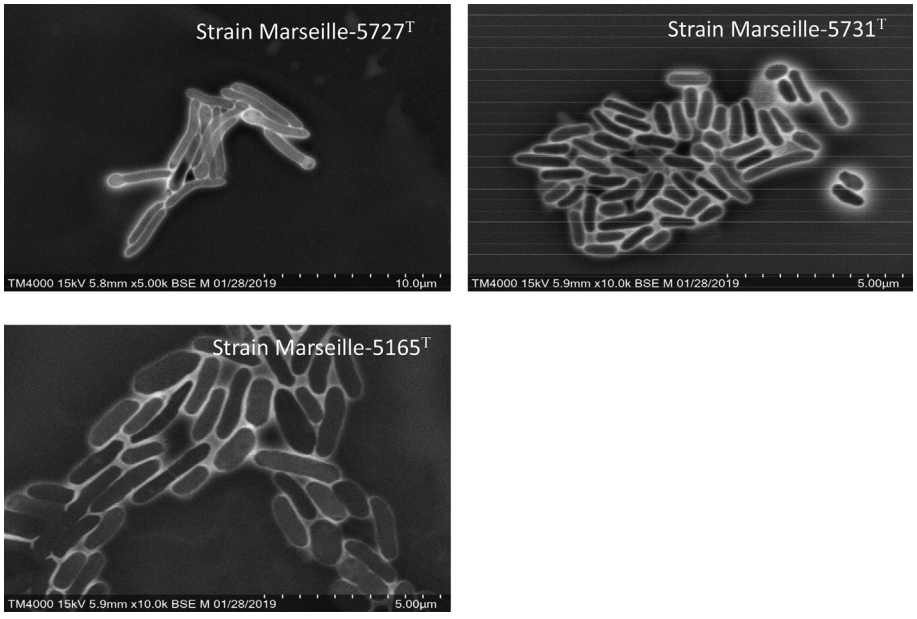


**FIG. 3.** Electron micrograph of *Lysinibacillus timonense* Strain Marseille-P5727$^T$ *Microbacterium timonense* Strain Marseille-P5731$^T$, *Erwinia mediterraneensis* Strain Marseille-P5165$^T$ was acquired with a Hitachi TM4000Plus tabletop scanning electron microscope (SEM). A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 min and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water; air-dried and examined in a tabletop SEM (Hitachi TM4000). Scales and acquisition settings are shown in the figure.

*trichothecenolyticum*. When *Microbacterium timonense* was compared with these closely related species, values ranged from 73.74% with *Microbacterium oleivorans* to 75.31% with *Microbacterium hydrocarbonoxydans*.

The genome of strain Marseille-P5165$^T$ was 4 275 595 bp long with a 54.9% G+C content. OrthoANI values among closely related species ranged from 62.76% between *Erwinia insecta* and *Erwinia oleae* to 79.34% between *Pantoea agglomerans* and *Pantoea cypripedii*. When *Erwinia mediterraneensis* was compared with these closely related species, values ranged from 74.94% with *Erwinia oleae* to 79.02% with *Pantoea agglomerans* (Fig. 4).

## Conclusion

Based on the results for unique phenotypic characteristics, including MALDI-TOF spectrum, a 16S rRNA sequence divergence >1.3% and an OrthoANI value <95% with the phylogenetically closest species with standing in nomenclature, we formally proposed strains Marseille-P5727$^T$, Marseille-P5731$^T$ and Marseille-P5165$^T$, respectively, as being the type strains of *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., and *Erwinia mediterraneensis* sp. nov., which are new species in the Bacteria domain.

### Description of *Lysinibacillus timonensis* sp. nov.

*Lysinibacillus timonensis* (ti.mon.en'sis. N.L. masc. adj. *timonensis* the name of quarter La Timone where the strain was isolated). Isolated from the palm of the hand of a healthy person living in rural Senegal. *Lysinibacillus timonensis* is Gram-negative, aerobic and spore-forming; it is a club-shaped rod and showed catalase-positive and oxidase-negative activities. The strain develops readily on Chapman–mannitol salt agar with aerobic and non-mobile cells with a mean length of 3.74 µm and a mean width of 0.61 µm. The G+C content of the genome is 70.1mol%. The 16S rRNA and genome sequences of *L. timonensis* strain Marseille-P5727$^T$ (CSURP5727) are deposited in GenBank under Accession numbers LT985390 and OLMT00000000, respectively.

### Description of *Microbacterium timonense* sp. nov.

*Microbacterium timonense* (ti.mo.nen'se N.L. neut. adj. *timonense*, pertaining to La Timone the name of the hospital in Marseille, France, where the first strains were isolated). Isolated from the palm of the hand of healthy person living in rural Senegal, *M. timonense* strain Marseille-P5731$^T$ is Gram-negative, non-spore-forming and showed catalase activity, but not oxidase activity. The strain develops readily on 5% Columbia agar enriched with sheep blood with aerobic and mobile cells with a mean length of 1.25 µm and a mean width of 0.53 µm. The G+C content of the genome is 70.1%. The 16S rRNA and genome

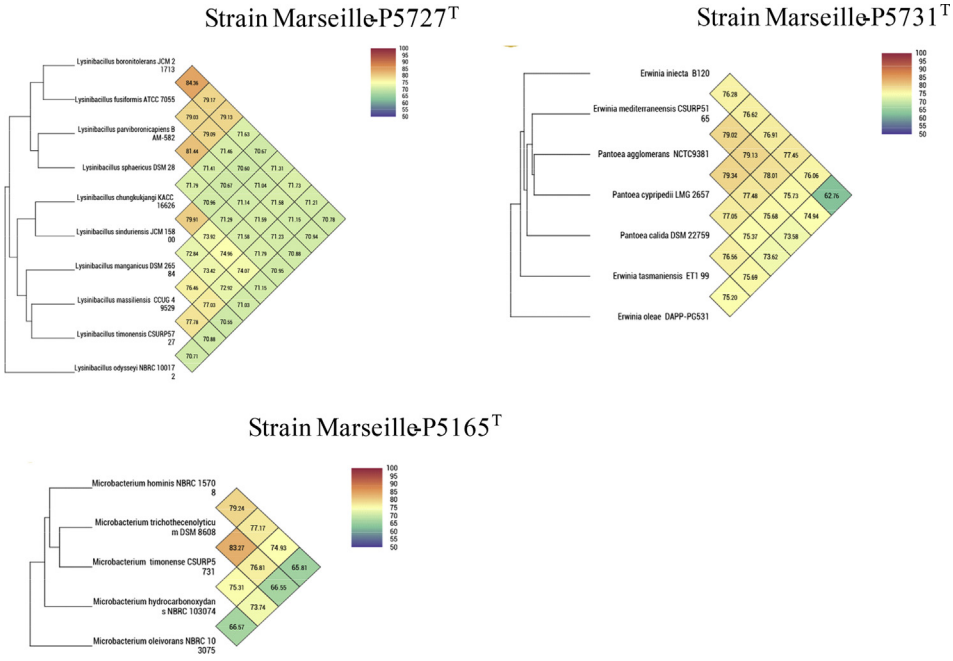


FIG. 4. Heatmap generated with OrthoANI values calculated using the OAT software between *Lysinibacillus timonensis* sp. nov., *Microbacterium timonense* sp. nov., *Erwinia mediterraneensis* sp. nov. and other closely related species with standing in nomenclature.

sequences of *M. timonense* strain Marseille-P5731$^T$ (CSURP5731) are deposited in GenBank under Accession numbers LT985453 and OLMV00000000, respectively.

**Description of *Erwinia mediterraneensis* sp. nov.**

*Erwinia mediterraneensis* (me.di.ter.ra.ne.en' sis, L. masc. adj., *mediterraneensis* of Medi-terraneum, the Latin name of the Mediterranean Sea by which Marseille is located, and where strain P5165 was isolated). They are Gram-negative, motile and have mean length of 1.98 µm and mean width of 0.62 µm. Strain Marseille-P5165$^T$ shows catalase and oxidase activities. The strain develops readily on 5% Columbia agar enriched with sheep blood. The G+C content of the genome is 54.9%. The 16S rRNA and genome sequences of *E. mediterraneensis* strain Marseille-P5165$^T$ (CSURP5165) are deposited in GenBank under accession numbers LT996111 and UWOB00000000, respectively.

*Nucleotide sequence accession number.* The 16S rRNA gene and genome sequences of *Lysinibacillus timonensis* were deposited in GenBank under Accession numbers LT985390 and OLMT00000000, respectively. The 16S rRNA gene and genome sequences of *Microbacterium timonense* were deposited in GenBank under Accession numbers LT985453 and OLMV00000000, respectively. The 16S rRNA gene and genome sequences of *Erwinia mediterraneensis* were deposited in GenBank under Accession numbers LT996111 and UWOB00000000, respectively.

*Deposit in culture collection.* Strain Marseille-P5727$^T$, strain Marseille-P5731$^T$ and strain Marseille-P5165$^T$ were deposited in the Collection de Souches de l'Unité des Rickettsies (CSUR) under the following numbers, respectively: CSURP5727, CSURP5731 and CSURP5165.

## Conflict of interest

None to declare.

## Acknowledgements

The authors thank Catherine Robert for sequencing the genome, Aurelia Caputo for submitting the genomic sequence to GenBank, and Carine Couderc for performing the MALDI-TOF reference spectrum.

## Funding sources

This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the programme *Investissements d'avenir*, reference ANR-10-IAHU-03.

## Ethics and consent

The study and consent procedures were approved by the Senegalese Comité National d'Ethique pour la Recherche en Santé, ethics committee in accordance with the SEN protocol No. 53/MSAS/DPRS/CNERS on 31 March 2015 as well as by the ethics committee of the Institut Hospitalo-Universitaire Méditerranée Infection. The volunteers gave written consent.

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Article 6

# *Corynebacterium senegalense* sp. nov. and *Arthrobacter senegalensis* sp. nov., two new Actinobacteria isolated from skin swab from the palm of hand

C. Ndiaye, N. S. Fall, E. Kuete, C. I. Lo, D. Raoult, C. Sokhna and J.-C. Lagier

Publié dans New Microbes and New Infections

# *Corynebacterium senegalense* sp. nov. and *Arthrobacter senegalensis* sp. nov., two new *Actinobacteria* isolated from skin swab from the palm of hand

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## Abstract

*Corynebacterium senegalense* strain Marseille-P4329$^T$ (= CSURP4329) and *Arthrobacter senegalensis* strain Marseille-P4329$^T$ (= CSURP4198) are new species first isolated from human skin. A culturomics approach and taxonogenomics methods were used for these new bacterial species.


**Keywords:** *Arthrobacter senegalensis* sp. nov., *Corynebacterium senegalense* sp. nov., culturomics, human skin, taxonogenomics
**Original Submission:** 7 May 2019; **Accepted:** 24 June 2019
**Article published online:** 28 June 2019

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## Introduction

The skin microbiota has great importance for human health. It is involved in many cutaneous diseases and plays a vital role in wound infections [1]. Most bacteria living on the skin belong to three phyla: *Actinobacteria*, *Firmicutes* and *Proteobacteria* [2]. *Actinobacteria* phylum regroup most of bacterial species located in different sites on the skin [3]. The genus *Arthrobacter* is known as a member of the skin flora, moreover *Arthrobacter mysorens* has been implicated in localized skin infection [4]. In contrast, the genus *Corynebacterium* is a commensal of the skin and is one of the most commonly isolated *Actinobacteria* [4,5].

Implications of bacterial diversity in normal physiological functions and susceptibility to diseases has become a crucial topic to explore [6]. In our laboratory we adopt a new approach called culturomics that isolates bacteria under various culture conditions while associating identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and 16S rRNA amplicon sequencing to study the diversity of human bacteria [7–9]. The new species that we present here—*Corynebacterium senegalense* sp. nov. and *Arthrobacter senegalensis* sp. nov.—have been described using a combination of genotypic and phenotypic characteristics, following a taxonogenomic strategy previously described [10,11].

## Isolation and growth conditions

In 2017, unidentified bacterial strains were isolated from the palm of hand of two healthy persons living in Ndiop, in rural Senegal. The study was validated by the ethics committee of Senegal (No. 53/MSAS/DPRS/CNERS du 31 mars 2015). Initial growth of *Corynebacterium senegalense* and *Arthrobacter senegalensis* was obtained after 24 h of incubation at 37°C on Columbia Colistin and Nalidixic Acid +5% sheep blood (BioMérieux, Marcy l'Etoile, France) under aerobic conditions at pH 7.3. Samples were screened using MALDI-TOF MS on a Microflex LT spectrometer (Bruker Daltonics, Bremen, Germany) as previously described [12]. The obtained spectra (Fig. 1) were imported into MALDI BIOTYPER 3.0 software (Bruker Daltonics) and analysed against the main spectra of the bacteria included in the database (Bruker database, constantly updated with MEPHI database).



https://doi.org/10.1016/j.nmni.2019.100583

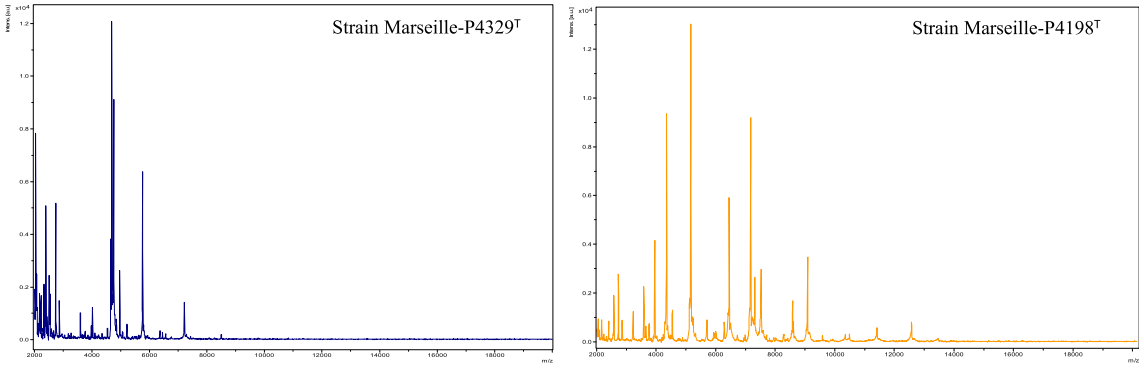


**FIG. 1.** MALDI-TOF MS reference spectrum of the two new species described. The reference spectra were generated by comparison of spectra from 12 individual colonies for each species.

## Strain identification

To classify these bacteria, the 16S rRNA gene was amplified using the primer pair fD1 and rP2 (Eurogentec, Angers, France) and sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit and 3500xL Genetic Analyzer capillary sequencer (Thermofisher, Saint-Aubin, France), as previously described [13]. The 16S rRNA nucleotide sequences were assembled and corrected using CodonCode Aligner software (http://www.codoncode.com). Strain Marseille-P4329$^T$ exhibited a 97.56% sequence identity with *Corynebacterium lipophiloflavum* strain DSM 44291 (GenBank Accession number: NR_026370.1), the phylogenetically closest species with standing in nomenclature (Fig. 2a). However, the strain Marseille-P4198$^T$ exhibited a 98.10% sequence identity with *Arthrobacter crystallopoietes* strain DSM 20117 (GenBank Accession number: NR_026189.1), the phylogenetically closest species with standing in nomenclature (Fig. 2b). We consequently classify these strains as members of new species within the phylum *Actinobacteria*.

## Phenotypic characteristics

*Corynebacterium senegalense* strain Marseille-P4329$^T$ colonies were yellow, circular and shiny with a smooth surface. Bacterial cells were Gram-positive, non-motile, non-spore-forming, club-shaped rods, 0.9 µm long and 0.6 µm wide. Strain Marseille-P4329$^T$ showed catalase-positive and oxidase-negative activities. *Arthrobacter senegalensis* strain Marseille-P4198$^T$ colonies were grey with irregular edges. Bacterial cells were Gram-negative, motile, 1.2 µm long and 0.5 µm wide. Strain Marseille-P4198$^T$ showed catalase-positive and oxidase-negative activities (Fig. 3).

## Genome sequencing

Genomic DNA was extracted using the EZ1 biorobot (Qiagen, Courtaboeuf, France) with the EZ1 DNA tissue kit and then sequenced on the MiSeq technology (Illumina, San Diego, CA, USA) with the Nextera Mate Pair sample prep kit and Nextera XT Paired end (Illumina), as previously described [14]. The assembly was performed with a pipeline incorporating different softwares (Velvet [15], Spades [16] and Soap Denovo [17], on trimmed data (MiSeq and Trimmomatic [18] softwares) or untrimmed data (only MiSeq software). GapCloser was used to reduce assembly gaps. Scaffolds <800 bp and scaffolds with a depth value < 25% of the mean depth were removed. The best assembly was selected by using different criteria (number of scaffolds, N50, number of N). The degree of genomic similarity of these two strains with their closely related species was estimated using OrthoANI software [19]. The genome strain Marseille-P4329$^T$ is 2 311 841 bp long with 52.8% G+C content. OrthoANI values among closely related species ranged from 68.48% between *Corynebacterium terpenotabidum* and *Corynebacterium casei* to 82.08% between *Corynebacterium senegalense* and *Corynebacterium timonense*. When *Corynebacterium senegalense* was compared with these closely related species, values ranged from 69.61% with *Corynebacterium casei* to 82.08% with *Corynebacterium timonense* .

The genome of strain Marseille-P4198$^T$ had a length of 4 027 190 bp with 66.1% G+C content. OrthoANI values among closely related species ranged from 64.80% between

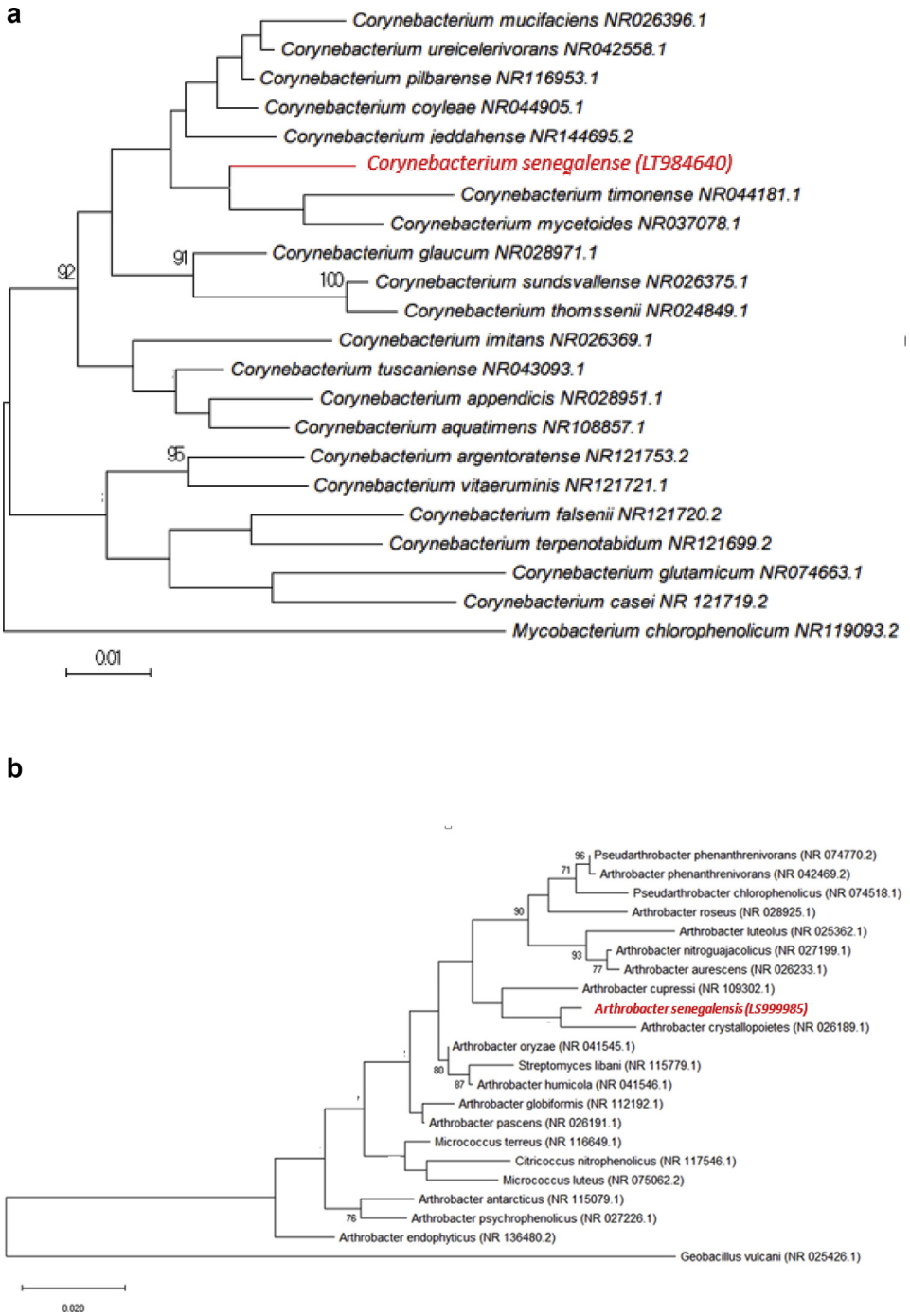


**FIG. 2.** (a) Phylogenetic tree showing the position of *Corynebacterium senegalense* strain Marseille-CSURP4329$^T$ relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters, and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 500 times to generate a majority consensus tree. The scale bar indicates a 1% nucleotide sequence divergence. (b) Phylogenetic tree showing the position of *Arthrobacter senegalensis* strain Marseille-CSURP4198$^T$ relative to other phylogenetically close neighbours. The respective GenBank accession numbers for 16S rRNA genes are indicated in parenthesis. Sequences were aligned using MUSCLE v3.8.31 with default parameters, and phylogenetic inferences were obtained using the maximum likelihood method within MEGA 7 software. Numbers at the nodes are percentages of bootstrap values obtained by repeating the analysis 1000 times to generate a majority consensus tree. The scale bar indicates a 2% nucleotide sequence divergence.

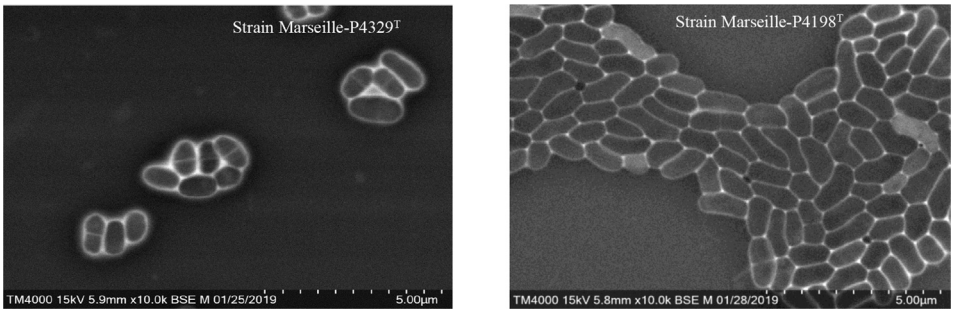


**FIG. 3.** Electron micrographs of *Corynebacterium senegalense* Strain Marseille-P4329$^T$ *Arthrobacter senegalensis* strain Marseille-P4198$^T$ were acquired with a Hitachi TM4000Plus tabletop scanning electron microscope (SEM). A colony was collected from agar and immersed into a 2.5% glutaraldehyde fixative solution. Then a drop of the suspension was directly deposited on a poly-L-lysine-coated microscope slide for 5 minutes and treated with 1% phosphotungstic acid aqueous solution (pH 2.0) for 2 minutes to increase SEM image contrast. The slide was gently washed in water, air-dried and examined in a tabletop SEM (Hitachi TM4000) approximately 60 cm in height and 33 cm in width to evaluate bacterial structure. Scales and acquisition settings are shown in the figure.

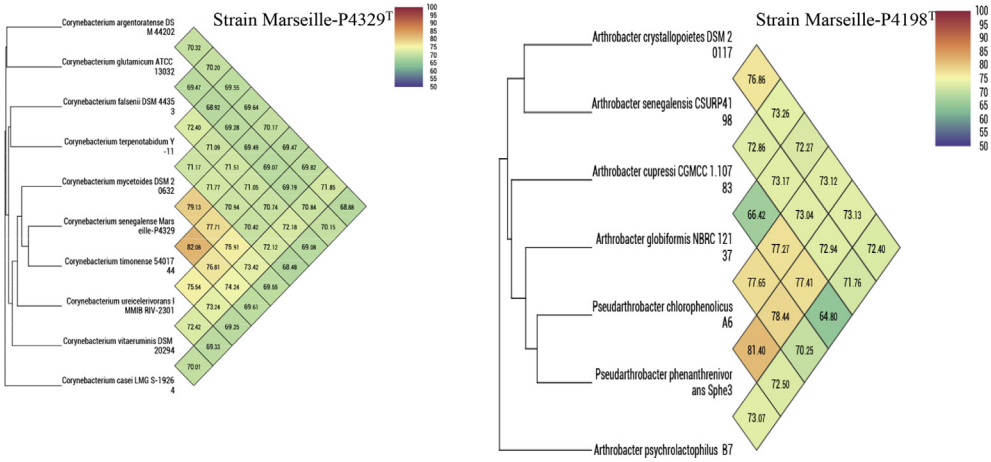


**FIG. 4.** Heatmap generated with OrthoANI values calculated using the OAT software between *Corynebacterium senegalense* sp. nov., *Arthrobacter senegalensis* sp. nov. and other closely related species with standing in nomenclature.

*Arthrobacter cupressi* and *Arthrobacter psychrolactophilus* to 81.40% between *Pseudoarthrobacter chlorophenolicus* and *Pseudoarthrobacter phenanthrenivorans*. When *Arthrobacter senegalensis* was compared with these closely related species, values ranged from 71.76% with *Arthrobacter psychrolactophilus* to 76.86% with *Arthrobacter crystallopoietes* (Fig. 4).

## Conclusion

Strain Marseille-P4329$^T$ and strain Marseille-P4198$^T$ exhibited 16S rRNA sequence divergence >1.3% and OrthoANI values < 95% with its phylogenetically closest species with standing in nomenclature, together with unique phenotypic features. Based on these results, we consequently proposed the strains Marseille-P4329$^T$ and Marseille-P4198$^T$ as, respectively, the type strains of *Corynebacterium senegalense* sp. nov. and *Arthrobacter senegalensis* sp. nov.

## Description of *Corynebacterium senegalense* sp. nov.

*Corynebacterium senegalense* (se.ne.ga.len'se, L. fem. adj. *senegalense* related to Senegal, the name of the country where the sample was collected). Isolated from the palm of the hand of a healthy person living in rural Senegal. *Corynebacterium senegalense* is a Gram-positive, aerobic, non-spore-forming, club-shaped rod and showed catalase-positive and oxidase-negative activities.

The strain develops readily on Columbia agar enriched with 5% sheep blood with aerobic and non-mobile cells with a mean length of 0.965 µm and a mean width of 0.654 µm. The G+C content of the genome is 52.8%. The 16S rRNA and genome sequences of *C. senegalense* strain Marseille-P4329$^T$ (CSURP4329) are deposited in GenBank under Accession numbers LT984640 and OVSI00000000, respectively.

## Description of *Arthrobacter senegalensis* sp. nov.

*Arthrobacter senegalensis* (se.ne.ga.len'sis, L. masc. adj. *senegalensis* related to Senegal, the name of the country where the sample was collected). Isolated from the palm of the hand of a healthy person living in rural Senegal. *Arthrobacter senegalensis* is Gram-negative, aerobic, non-spore-forming, with irregular edges, and showed catalase-positive and oxidase-negative activities.

The strain develops readily on Columbia agar enriched with 5% sheep blood with aerobic and non-mobile cells with a mean length of 1.250 µm and a mean width of 0.530 µm. The G+C content of the genome is 66.1%. The 16S rRNA and genome sequences of *A. senegalensis* strain Marseille-P4198$^T$ (CSURP4198) are deposited in GenBank under Accession numbers LS999985 and PRJEB28787, respectively.

### Nucleotide sequence accession number

The 16S rRNA gene and genome sequences of *Corynebacterium senegalense* sp. nov. were deposited in GenBank under Accession numbers LT984640 and OVSI00000000, respectively.

The 16S rRNA gene and genome sequences of *Arthrobacter senegalensis* sp. nov. were deposited in Genbank under accession number LS999985 and PRJEB28787 respectively.

### Deposit in culture collection

Strain Marseille-P4329$^T$ and Strain Marseille-P4198$^T$ were deposited in the CSUR collection under the numbers (= CSURP4329) and (= CSURP4198), respectively.

## Acknowledgements

The authors thank Aurelia Caputo for submitting the genomic sequence to GenBank.

## Conflicts of interest

None to declare.

## Funding sources

This study was supported by the Institut Hospitalo-Universitaire (IHU) Méditerranée Infection, the National Research Agency under the programme Investissements d'avenir, reference ANR-10-IAHU-03.

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Article 7

# ‘*Citricoccus massiliensis’* sp. nov., a new bacterial species isolated from human skin by culturomics

C. Ndiaye, H. Bassène, F. Cadoret, D. Raoult, J. C. Lagier and C. Sokhna

Publié dans New Microbes and New Infections

# *'Citricoccus massiliensis'* sp. nov., a new bacterial species isolated from human skin by culturomics

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## Abstract

We report the main characteristics of '*Citricoccus massiliensis*'strain Marseille-P4330, a new species within the genus *Citricoccus.* This strain was isolated from the skin of a healthy human man living in Dielmo, Senegal, Western Africa.


**Keywords:** *Citricoccus massiliensis*, culturomics, human microbiota, human skin
**Original Submission:** 29 December 2017; **Revised Submission:** 31 January 2018; **Accepted:** 9 February 2018
**Article published online:** 17 February 2018

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Skin was thought to harbour a limited number of microbial communities, primarily composed of aerobic cocci. Genomic studies have offered additional insight into the diversity of skin microbiota and have also allowed for quantitative analyses [1]. The earliest descriptions of skin microbiota were based on culture-dependent studies. In our laboratory, a culturomics approach, consisting of rapid identification of grown colonies using matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), has been developed to isolate previously uncultured human bacteria [2,3]. We used this technique to explore the human skin bacterial repertoire from skin swabs collected from healthy patients living in rural Senegal. The project was approved by the National Ethics Committee of Senegal (no. 53/MSAS/DPRS/CNERS, 31 March 2015).

In 2017, we isolated from skin swabs a bacterial strain that could not be identified by our MALDI-TOF MS screening on a Microflex spectrometer (Bruker Daltonics, Bremen, Germany) [4]. The 16S rRNA gene was sequenced using fD1-rP2 primers as previously described using a 3130-XL sequencer (Applied Biosciences, Saint-Aubin, France) [5]. Strain Marseille-P4330 exhibited a 98.61% sequence identity with *Citricoccus nitrophenolicus* strain NPP1 (GenBank accession no. NR_112632) (Fig. 1). Because the strain showed a similarity of 16S rRNA gene sequence <98.65% with its phylogenetically closest species with standing in nomenclature, we here propose the creation of a new species [6].

Strain Marseille-P4330 was isolated in a skin swab from a 19-year-old man living in Dielmo, Senegal, Western Africa. The growth occurred in Columbia agar culture media with colistin and nalidixic acid (bioMérieux, Marcy l'Etoile, France) and 5% sheep's blood (bioMérieux). The initial agar-grown colonies were obtained after 24 to 48 hours' incubation at 37°C. Colonies were yellowish, circular, shiny, smooth and convex with regular edges and presented a mean diameter of 1.2 mm. The strain was an aerobic, Gram-positive, spore-forming and motile coccus-shaped bacterium, with positive catalase reaction and negative oxidase reaction.

Thus, we propose to classify strain Marseille-P4330 as a new species of the genus *Citricoccus* within the family *Micrococcaceae,* phylum *Actinobacteria* (GenBank accession no. LT960590).

## Description of new species '*Citricoccus massiliensis*'

'*Citricoccus massiliensis*' had yellowish, circular, shiny, smooth and convex colonies with a mean diameter of 1.2 mm. The



https://doi.org/10.1016/j.nmni.2018.02.004

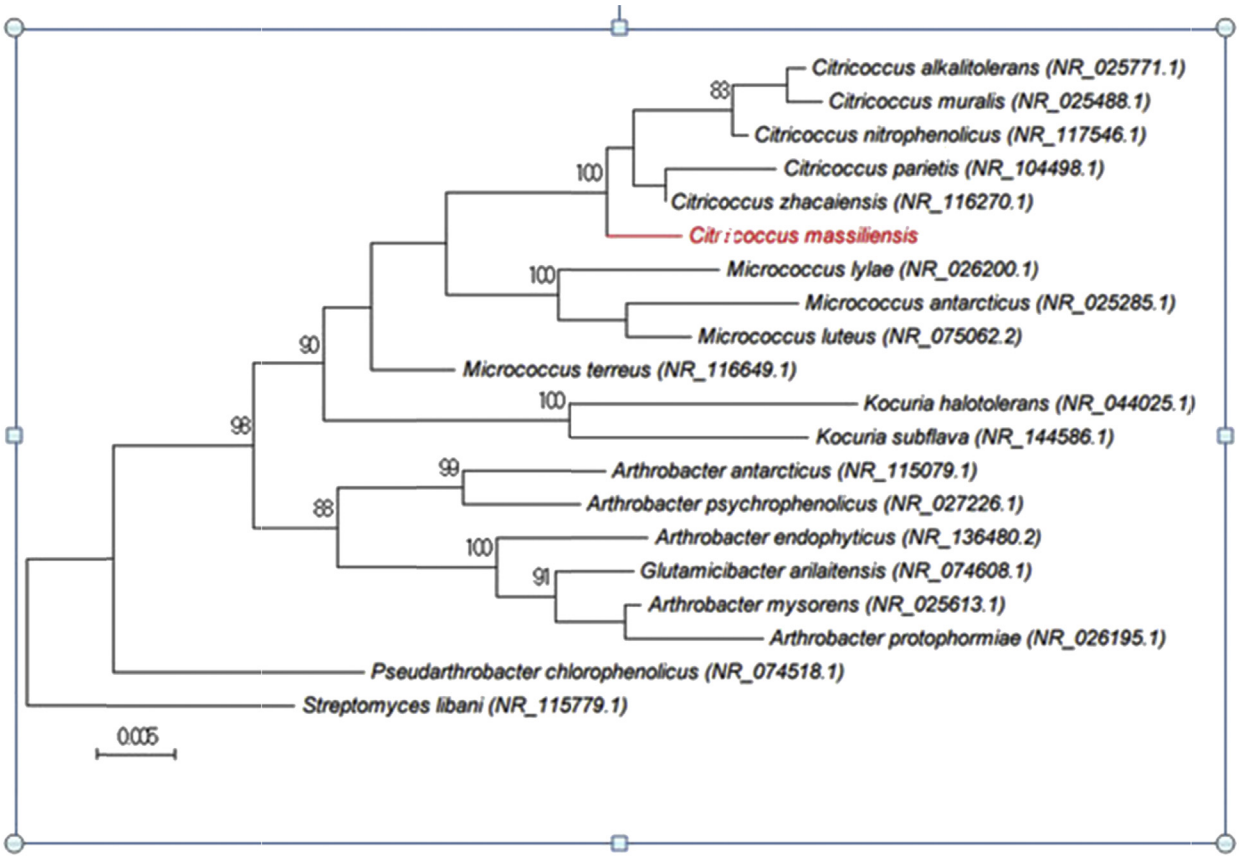


**FIG. 1.** Phylogenetic tree showing position of *'Citricoccus massiliensis'* Marseille-P4330 relative to other phylogenetically close neighbours. 16S rRNA gene sequences were aligned using CLUSTAL W, and phylogenetic inferences were obtained using maximum-likelihood method within MEGA software. Numbers at nodes are percentages of bootstrap values obtained by repeating analysis 500 times to generate majority consensus tree. Scale bar indicates 0.5% of nucleotide sequence divergence.

strain was an aerobic, Gram-positive, spore-forming and motile coccus-shaped bacterium, with positive catalase reaction and negative oxidase reaction. Strain Marseille-P4330$^T$ (= CSUR P4330) is the type strain of the new species '*Citricoccus massiliensis*' (ma.ssi.li.en'sis, L. masc. adj. *massiliensis,* pertaining to Massilia, the Roman name of Marseille, where the strain was isolated).

## Nucleotide sequence accession number

The 16S r-RNA gene sequence was deposited in GenBank under accession number LT960590.

## Deposit in a culture collection

Strain Marseille-P4330 was deposited in the Collection de souches de l'Unité des Rickettsies (CSUR, WDCM 875) under number P4330.

Acknowledgements.

This study was funded by the Fondation Méditerranée Infection. We thank M. D. Mbogning Fonkou for technical assistance.

## Conflict of interest

None declared.

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# Conclusion et perspectives

Les deux premiers chapitres de ce travail ont permis d'établir le répertoire des bactéries isolées par culture au moins une fois à partir d'un écouvillon cutané chez des sujets africains généralement en bonne santé, ce qui permet de constituer un repère pour la communauté scientifique. Nous avons ainsi, en utilisant la culturomics, démontré sa capacité à isoler un grand nombre de bactéries variées, en comprenant des espèces non humaines et des nouvelles espèces. Ceci permet de dire que les méthodes de culture sont indispensables dans l'exploration du microbiome humain considérant que nous n'avons qu'une vision partielle des communautés bactériennes colonisant les différents sites anatomiques de l'homme.

En perspective, une étude de culture comparative chez des sujets en bonne santé et chez des sujets avec certaines affections dermatologiques telles que l'eczéma, le psoriasis, l'acné la rosacée... pourrait être réalisée pour voir si des microbes spécifiques ou des changements de la communauté microbienne sur la peau initient ou maintiennent certaines de ces maladies. L'identification de ces changements dynamiques peut être utile à la fois comme biomarqueur et comme cible thérapeutique potentielle. Nous proposons une extension de l'exploration du microbiote cutané à d'autres microorganismes associés à l'homme tels que les archées, les virus, les parasites et les champignons. Faire un suivi dynamique sur une période de longue durée et y associer d'autres sites de la peau pour répondre à la question de la contamination manuportée. Ainsi, nous pensons que la combinaison de méthodes multi-omiques que la culturomique et la métagénomique et la protéomique sera plus appropriée pour une étude très poussée du microbiote cutané. Elle améliore considérablement notre capacité à comprendre la structure et fonction du microbiote tant chez les individus avec des affections de la peau que dans les chez les individus sains.

Les chapitres trois et quatre de ce travail nous a permis de démontrer l'existence réelle de certaines bactéries pathogènes sur la peau par la méthode moléculaire de détection la qPCR

chez une population généralement en bonne santé. Mais également ils démontrent l'efficacité de la promotion de l'hygiène corporelle dans la réduction du portage cutanée de certaines bactéries pathogènes voir plus loin signes d'infections respiratoires tels que la toux, l'écoulement nasal et les fièvres. Cette étude montre qu'une stratégie simple et peu coûteuse basée sur le lavage des mains et du corps avec de l'eau et du savon pourrait réduire de manière significative l'incidence des infections respiratoires, des maladies fébriles et du développement des bactéries pathogènes. L'efficacité du lavage des mains au savon sur la diarrhée n'a pas été démontrée dans cette étude. Des mesures supplémentaires, telles qu'une bonne sensibilisation du public, la prise en charge des enfants mal nourris, l'augmentation de l'approvisionnement en eau potable, etc. doivent être associées au lavage des mains pour réduire les taux d'incidence. Sur la base de ces résultats, il semble nécessaire d'entreprendre une étude évaluant la durabilité du changement de comportement dans la promotion du lavage des mains, et il est également important d'évaluer le rapport coût-efficacité. Enfin, les autorités sanitaires du pays devraient tenir compte de ces résultats et promouvoir le lavage des mains à l'eau et au savon dans leurs programmes de prévention des maladies infectieuses.

Nous proposons aussi une extension de l'étude sur d'autres microorganismes tels que les virus les champignons et les parasites, surtout ces derniers qui sont souvent causes de maladies diarrhéiques parasitaires qui sont souvent due à un manque d'hygiène, d'eau potable et l'absence de latrine. Mais également d'élargir l'étude en milieu urbaine avec une forte démographie et les inégalités de développement sont très présentes et la majeure partie prenne leur repas dans les restaurants à moitié couvert avec une promiscuité énorme et le plus souvent les mesures d'hygiène ne sont pas respectées.

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