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Original article

Genetic Determinism in Community *Staphylococcus* and Methicillin-Resistant Clinics in Brazzaville, Republic of Congo

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Abstract

In order to demonstrate the transmission of resistance genes between clinical bacteria and community bacteria, 78 strains of *Staphylococcus* including 56 (71.79%) community strains and 22 (28.21%) clinical strains were isolated and identified according to classical methods of microbiology. The resistance pattern was determined by the standard Mueller Hinton diffusion method. The phenotype of methicillin-resistant *Staphylococci* was investigated from the oxacillin disc and cefoxitin. PCRs were performed on 45 DNA strains of *Staphylococcus* including 25 (55.56%) of community strains and 20 (44.44%) of clinical strains resistant to oxacillin and cefoxitin alone or associated. Phenotypic results indicate that norfloxacin, ciprofloxacin, tobramycin, kanamycine were more active on community Staphylococci. In clinical *Staphylococcus*, only tobramycin was more active. The differences were significant between the resistance frequencies of community and clinical Staphylococci for some antibiotics with a P value <0.05. The mec A gene was identified in 9 community *S.aureus* strains, 6 clinical strains of *Staphylococcus*. The fragments of the amplified gene were of the same molecular weight (500bp), which suggests a spread of clinical strains in the city.

Keywords: ***Determinism, genetic, Staphylococcus, methicillin.***

1. Introduction

Staphylococcal infections are observed in multiple clinical situations, both in community pathology and nosocomial pathology. *Staphylococcus aureus* is considered to be an important cause of a wide variety of diseases in humans, such as: food poisoning, pneumonia, wounds and nosocomial infections[1],[2]. There are many anti-staphylococcal agents; however, the bacterium has developed mechanisms to neutralize them such as the mechanism of resistance to méthicilline[3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a cause of healthcare-associated infections[1], the community[2] and associated with livestock worldwide[4]. The treatment of infections has become increasingly difficult due to the high prevalence of methicillin-resistant strains and the development of the emergence of multidrug-resistant strains in different families of antibiotics. This phenomenon is observed at the hospital but also in the city[5]. MRSA (methicillinoresistance) infections exacerbate the prognosis and severely complicate the management of staphylococcal infections, which is a major public health problem[6],[7]. In the United States, about 29% (78.9 million people) and 1.5% (4.1 million) of the population have *S. aureus* and MRSA, respectively city[8]. An estimated 478,000 hospitalizations were associated with *S. aureus* infections, including 278,000 hospitalizations in 2005[9]. This situation led us to study the current state of this microorganism in Congo Brazzaville. Given that, little work has been done on this microorganism[10],[11]. Thus, this work aims to study the genetic determinism of resistance in *Staphylococcus* between community and clinical strains. By doing a comparative study of the resistance profiles as well as a PCR amplification of the mec A gene.

2. Material and Methods

1. Equipment

The biological material consisted of community strains isolated from household wastewater, biological products from outpatients

and isolated clinical strains from patients hospitalized at the Brazzaville University Hospital Center (CHUB).

2. Methods

2.1. Isolation and identification of strains

Isolated strains of wastewater were isolated from Chapman agar between July and September 2017. In outpatients the strains were isolated between January and March 2018 at the COGEMO clinic in Brazzaville. The clinical strains were isolated between January and July 2017 in the different departments of CHUB. The identification of community strains was made on the basis of culture traits on Chapman agar and biochemical (coagulase test) and by the API Staph gallery for clinical strains.

2.2. Antibiotic resistance

The resistance profile of bacterial strains has been evaluated by the standard antibiogram using the Mueller Hinton medium diffusion method[12],[13]. The antibiotics tested were the following: penicillin G (P., 10UI), oxacillin (OXA., 1µg), cefoxitin (CX., 30µg), vancomycin (VA., 30µg), Kanamycin (K., 5UI), gentamicin (CN., 10UI), tobramycin (TOB., 10µg), fosfomycin (FF., 200µg), ciprofloxacin (CIP., 5µg), norfloxacin (NOR., 10µg), erythromycin (E., 15µg), clindamycin (DA.,2 µg), pristinamycin (PT., 15 µg), fusidic acid (FA., 10 µg), rifampicin (RA., 5 µg). The antibiotic discs were applied to Mueller Hinton medium seeded by plating an inoculum prepared from a pure, young *Staphylococcus* colony. Antibiotic susceptibility was determined by the standard susceptibility testing method on Mueller Hinton medium. The resistance of Staphylococci to methicillin is investigated using oxacillin disks (OXA., 1 µg) in hypersaline Mueller Hinton medium (4% NaCl)[36] and cefoxitin (CX., 30 µg). After 18 to 24 hours of incubation in an oven at 37°C., the diffusion diameters of the various antibiotics were measured and compared with the reference diameters of the antibiogram committee of the French Microbiology Society[14].

2.3. Molecular detection of the mecA gene

1. DNA extraction

A total of 45 DNAs of *Staphylococcus* strains with a resistance phenotype of oxacillin and cefoxitin alone or associated were extracted by the nucleoSpin DNA Kit according to the MACHEREY NAGEL protocol.

2. Realization of the PCR

A PCR reaction was performed for amplification of the 310 bp fragment of the mecA gene using the primers; mec A-F: 5'-AAAATCGATGGTAAAGGTTGGC-3 'and mec A: 5'-AGTTCTGCAGTACCGGATTTGC-3[15],[16]. The PCR amplification reaction mixture (50 µl) contained 2 µl of DNA template and 48 µl of the mix. The mix consisted of 31.75 µl of sterile distilled water, 2 µl of F primer, 2 µl of R primer, 2 µl of dNTPs, 10 µl of PCR buffer (x5) and 0.25 µl of one taq. The PCR amplification conditions were as follows: initial denaturation at 94°C for 5 min, 30 denaturation cycles at 94°C for 30 sec, hybridization at 54°C for 30 sec, elongation at 72°C for 30 sec and final elongation at 72°C for 7 minutes.

3. Agarose gel electrophoresis of PCR products of the mec A gene

Five microliters (5µl) of PCR product were resolved on a 1.5% agarose gel containing 0.5 µg / ml of ethidium bromide in Tris-Borate-EDTA buffer at 100 V for 40 minutes.

2.4. Data processing

For the analysis of resistance data, intermediate category strains were counted as resistant (I + R). The data were analyzed using Graph Pad Prism 7 software. The Chi square test (X^2) was used to compare the resistance frequencies of community and clinical *Staphylococcus* strains. The 95% confidence interval and one degree of freedom of 1 were used. The difference between the frequencies was considered significant when the p value was less than 0.05.

3. Results

3.1. Isolation and identification of strains

In this study, 78 strains of *Staphylococcus* were isolated from household wastewater samples, biological products from outpatients and inpatients. Of these strains, 56 (71.79%) were community strains and 22 (28.21%) were clinical strains. Among community strains, we identified 49 (87.5%) strains of *S.aureus* and 7 (12.5%) strains of coagulase-negative *Staphylococcus* (SCN). The 22 clinical *Staphylococcus* strains consisted of 11 (50%) *S.aureus* strains and 11 (50%) SCN strains (Figure 1).

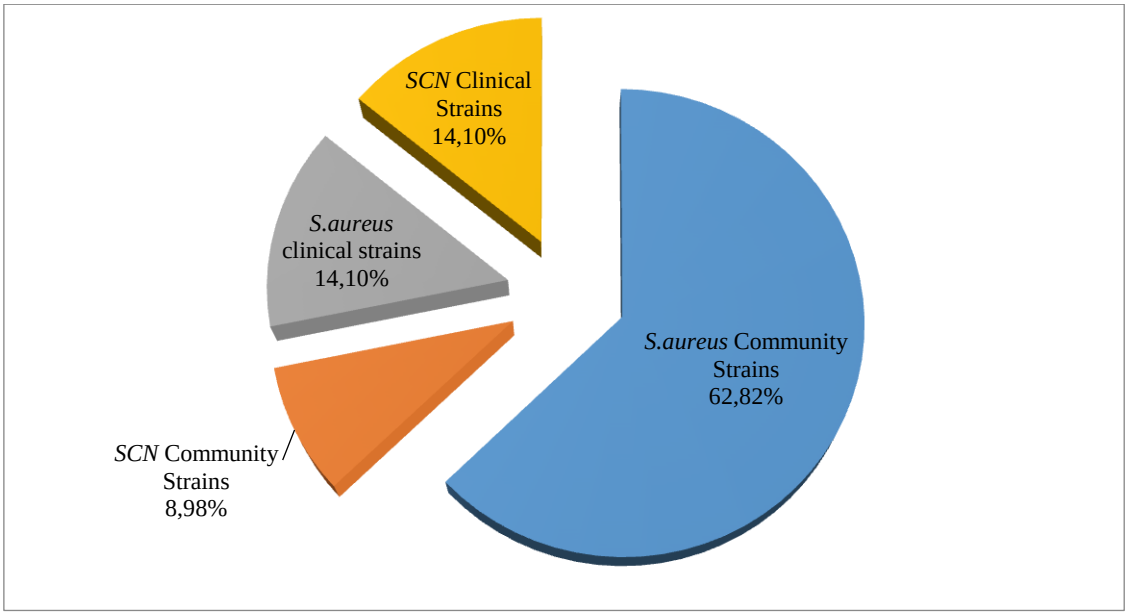
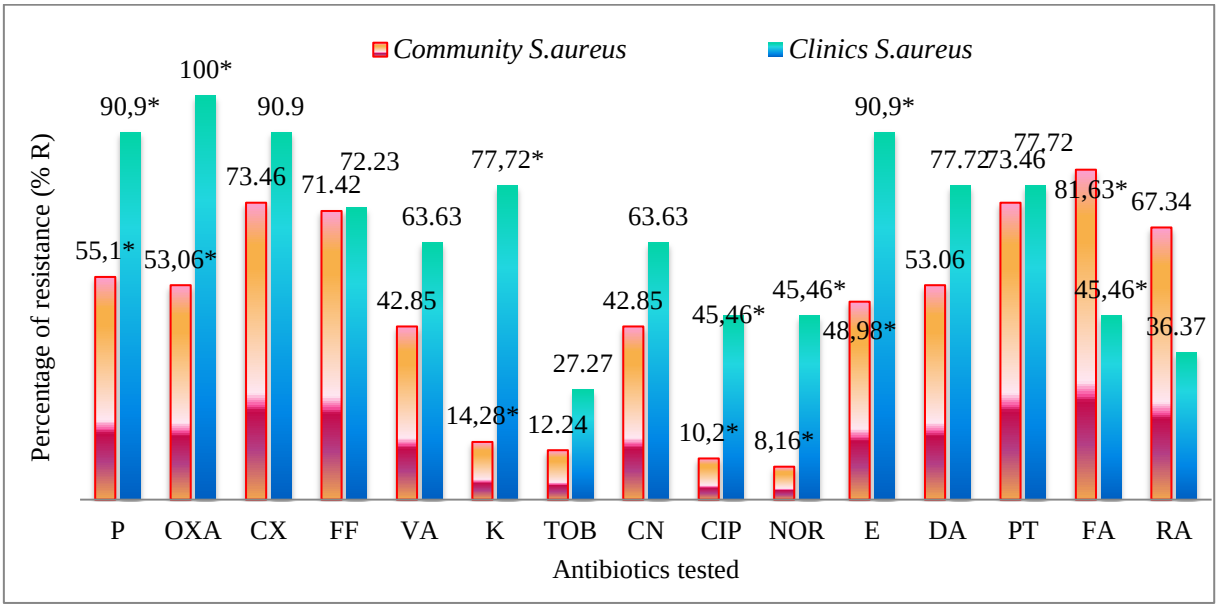


Figure 1: Distribution of *Staphylococcus* Strains

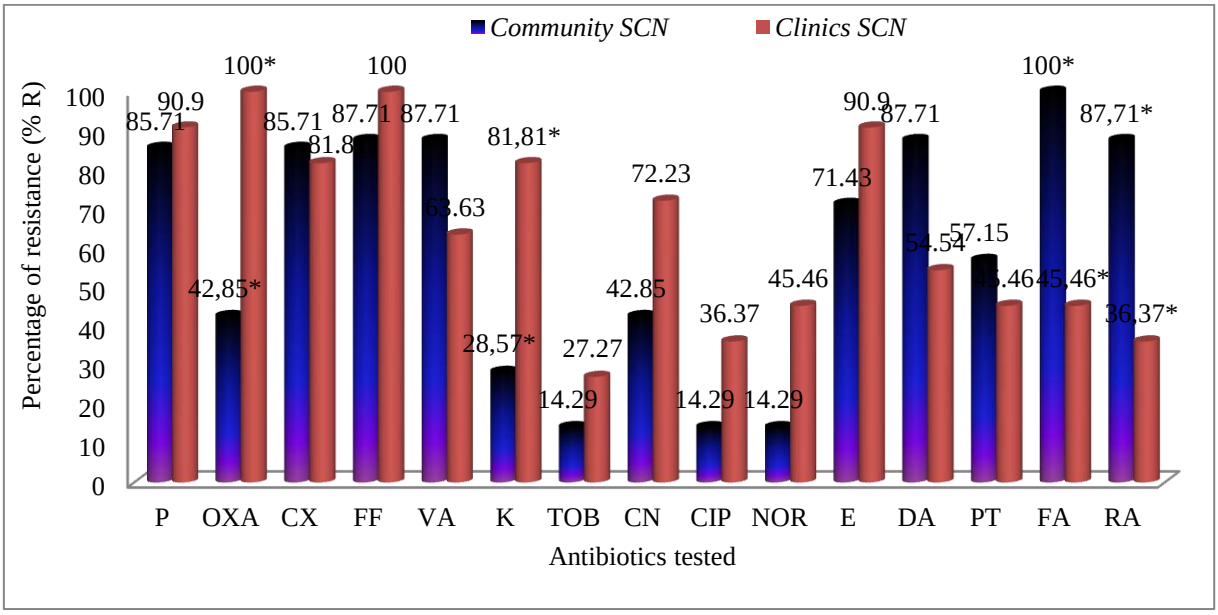
3.2. Phenotypic results

Figure 2 shows the different levels of resistance in community *S.aureus* and clinical *S. aureus*. Forty nine (49) *S. aureus* strains and eleven (11) clinical S. aureus strains were tested. In community *S. aureus*, the highest resistance was observed with fusidic acid (81.63%). Clinical *S. aureus* showed resistance ranging from 27.27% for tobramycin to 100% for oxacillin. The statistical test was significant for 7 (46.67%) antibiotics with a P value <0.05. In community *S. aureus*, the OXAS CXR phenotype (oxacillin susceptibility and cefoxitin resistance) was most strongly represented. The POXACX beta-lactam resistance phenotype (resistance to penicillin G, oxacillin and cefoxitin) was more represented in clinical *S. aureus*. In macrolide-lincosamide-Streptogramin (MLS) the EDAPT phenotype (resistance to erythromycin, clindamycin and pristinamycin) was more prevalent in community and clinical *S. aureus*.



*p <0.05

Figure 2: Resistance profile of community *S. aureus* and clinical *S. aureus* with antibiotics tested



*p <0.05

Figure 3: Resistance profile of community and clinical SCNs with tested antibiotics

Figure 3 represents the resistance profile of community and clinical SCNs. In community SCN, tobramycin, kanamycin, ciprofloxacin and norfloxacin were more active with respective percentages of resistance: 14.29%; 28.57% and 14.29%.

In clinical SCN, tobramycin was the only antibiotic with good activity at 27.27%. Resistance rates of 100% were observed for oxacillin and fosfomycin. The statistical test was significant for 4 (26.67%) antibiotics including oxacillin, kanamycin, fusidic acid and rifampicin with P value <0.05. The predominant beta-lactam resistance phenotypes were: P OXA CX (resistance to penicillin G, oxacillin and cefoxitin), PROXAR (resistance to penicillin G and oxacillin) in community SCN and P OXA CX at SCN Clinics. For aminoglycosides, the K TOB CN phenotype (resistance to kanamycin, tobramycin, and gentamycin) was predominant in community SCN and KCN (resistance to kanamycin and gentamycin) in clinical SCNs. In macrolides-lincosamides and streptogramins, the most prominent phenotypes were EDAPT (resistance to erythromycin, clindamycin and pristinamycin) and EDA (resistance to erythromycin and clindamycin) in community and clinical SCNs.

3.3. Genotypic results

The molecular study was performed on 45 DNA strains of *Staphylococcus*. These consist of 25 (55.56%) DNA from community strains and 20 (44.44%) DNA from clinical strains. The DNA of the community strains were composed of 19 (76%) *S. aureus* DNA and 6 (24%) DNA of SCN strains. The 20 DNA of the clinical strains were extracted from 11 (55%) *S.aureus* and 9 (45%) SCN. The mec A gene was identified in 9 (47.36%) community *S.aureus* strains (Figure 4) and strain 11 in Figure 5. Four (36.36%) clinical *S.aureus* strains and 2 (22,22%) Clinical SCN strains (Strains 18 and 22) illustrated in Figure 5 carry the mec A gene. The chi-square test was non-significant between the community and clinical *Staphylococcus* strains carrying the mecA gene. The fragments of the amplified gene were 500 bp.

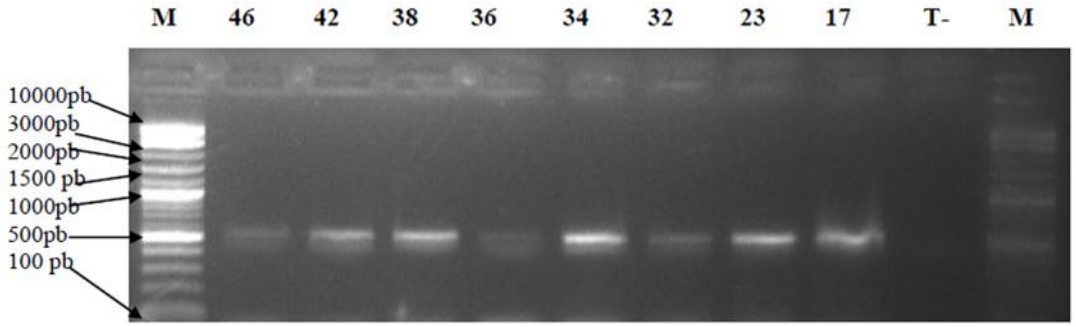


Figure 4: 1.5% Agarose gel electrophoresis of PCR amplicon of the mec A gene obtained with the DNA of *S. aureus* strains in the community; M = DNA lab marker; T = negative control; 17, 23, 32, 34, 36, 38, 42 and 46: fragments of community *S.aureus* strains.

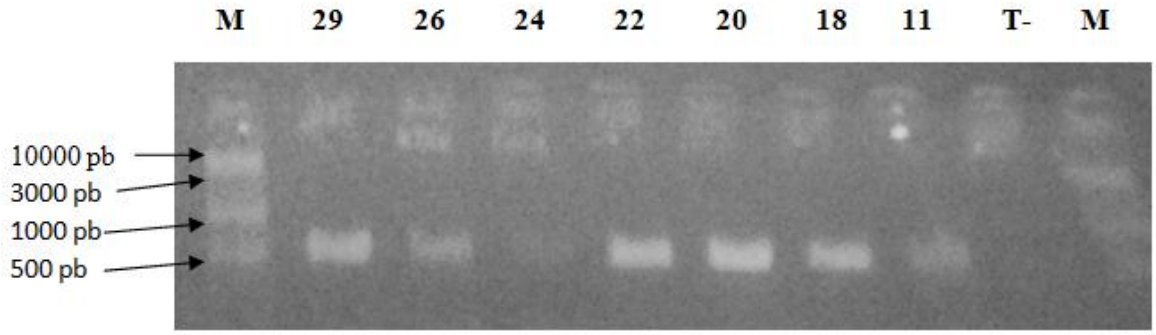


Figure 5: 1.5% Agarose gel electrophoresis of PCR amplicon of the mec A gene obtained with the DNA of *S.aureus* community strains, *S.aureus* and SCN clinics. M = DNA lab marker; T = negative control; 11: fragments of community *S.aureus* strains; 18, 22: fragments of clinical SCN strains and 20, 24, 26 and 29: fragments of clinical *S.aureus* strains.

4. Discussion

The study of the spread of drug resistance genes in Staphylococci is important to control its spread[21],[17]. The results of the resistance of community *S. aureus* obtained in our study reveal variable rates from one molecule to another. Resistance rates for penicillin G 55.10% (27), oxacillin 53.06% (26), kanamycin 14.26% (7), tobramycin 12.24% (6), gentamicin 42,55% (21) differ from those found by[11] in *S.aureus* isolated at Brazzaville University Hospital, whose respective percentages are: 100% (42), 92.86% (39), 97, 60% (41), 80.96% (34), 61.91% (26). Our clinical *S.aureus* results for the same antibiotics are: 90.90% (10), 100% (11), 77.72% (8), 27.27% (3) and 63.63% (7). These results corroborate those found by[11] Our resistance rates for penicillin G 90.90% (10), oxacillin 100% (11), kanamycin 81.81% (9), tobramycin 27.27% (3), gentamycin 72.23 % (8) at SCN clinics are quite close to those found by[11] whose respective percentages are: 100% (10), 10% (1), 90% (9), 60% (6), 30% (3). About 90% of *Staphylococcus* spp. Strains are currently resistant to penicillin G and penicillin A in France by penicillinase production[18]. The resistance of Staphylococci to penicillins M is secondary to a modification of the target, namely the synthesis of a penicillin binding protein PLP2a (or PLP2'), encoded by the mecA gene, and which has a decreased affinity for all beta-lactams[19]. Beta-lactam resistance in clinical *S.aureus* and SCN was predominantly dominated by the resistance phenotype P OXA CX. The resistance of *S. aureus* to penicillins M (oxacillin) is often associated with that of tobramycin and kanamycin. However, such strains generally remain susceptible to gentamicin[20]. This situation prevailed in our study where the K TOB CN phenotype was rarely encountered in 3 (15%) *S. aureus* strains and 1 (25%) community SCN. This phenotype has been absent in clinical *Staphylococcus*. Our phenotypic frequencies in community *S.aureus* and SCN differ from those found by[22]. Elsewhere, rates of 10% to 77% have been reported by[23],[24]. The main mechanism of resistance of *Staphylococcus* spp to aminoglycosides is enzymatic. The enzymes APH (3'), ANT (4') and APH (2') - AAC (6') respectively inactivate kanamycin (K phenotype), kanamycin and tobramycin (KTOB phenotype), and kanamycin, tobramycin and gentamicin (phenotype KTOBCN)[25]. Several genes for MLS resistance have been described in *S. aureus*. In France, the most frequent are ErmA, ErmB and ErmC[25]. Resistance to macrolide-streptogramins and lincosamides may also be due to msrA, a gene encoding a macrolide efflux protein[26]. The significant differences observed for penicillin, oxacillin, kanamycin, ciprofloxacin, norfloxacin, erythromycin, fusidic acid and rifampicin between community and clinical Staphylococci can be explained by the misuse of these molecules in a hospital setting. Molecular research of the mecA gene conducted during this study was performed on 25 (55.56%) DNA from community strains and 20 (44.44%) DNA from clinical strains. Nine (47.36%) community *S.aureus* strains, 4 (36.36%) clinical *S. aureus* strains and 2 (22.22%) clinical SCN strains carried the mec A gene. Methicillin from community and clinical strains was partially bound by an additional penicillin

binding protein (PLP2a). During our study, 3 strains of community *Staphylococcus* out of 25 strains resistant to oxacillin alone were selected. Two of them carried the mec gene A. Nine community strains were resistant to cefoxitin alone, 2 (22.22%) of them carried the mec A gene. All other community and clinical strains were both with oxacillin and cefoxitin. In a recent study, it was shown that a high frequency of *Staphylococcus* isolated from biomaterials contains the mec A gene[27]. In addition, we found 2 oxacillin-sensitive strains carrying the mec A gene. This result corroborates with those found by[28]. This is in contradiction with the results of[29]; they showed a complete agreement between the presence and absence of the mec A gene and the interpretation of the oxacillin disc susceptibility test. De Guisti et al.[30] suggested that A negative mock strains currently expressed phenotypic resistance to oxacillin mediated by a mechanism other than the presence of the mec A gene. Methicillin-resistant *S. aureus* are resistant to wide range of antimicrobial agents, including MLS, fluoroquinolones, tetracyclines, aminoglycosides and chloramphenicol[31]. This is in agreement with our results in which all the community strains carrying the mec A gene were resistant to MLS by presenting the E DA PT phenotype. Methicillin resistance mediated by the mec A gene as shown by[15],[16],[28] is consistent with our results. The mec A positive clinical strains exhibited the P OXA CX resistance phenotype to beta-lactams. This phenotype has been associated with the phenotypes E DA PT and E DA (resistance to erythromycin and clindamycin) to MLS. 42.85% of mecA positive clinical MRSA isolates presented the EDAPT resistance phenotype. This is greater than 6.09%, frequency reported by[16]. This frequency is lower than that reported by[32]. Several studies have been conducted on the molecular detection of the mec A gene in *Staphylococcus*. Our results are similar to those reported by several studies looking for the gene A gene in *Staphylococcus* but differ in the size of the genes obtained (500bp in our study). This size differs from that found by[15],[16] (310bp). This suggests that the amplified mec A gene in our study has several mutations. The presence of the mec A gene of the same molecular weight (500bp) in community *S.aureus, S.aureus* and clinical SCN underscores the extension of resistance genes within bacterial species from hospital to city. This is consistent with numerous resistance studies that have focused on MRSA as a nosocomial pathogen [33],[34],[28]. Layton et al.[35], Zmantar et al.[37] found that 28 to 41% of MRSA strains recovered from adult patients were of community origin.

5. Conclusion

Our study allows us to conclude that community and clinical *Staphylococcus* strains showed significant differences between the frequencies of resistance to a number of antibiotics. Norfloxacin, ciprofloxacin, tobramycin, kanamycin were more active on community Staphylococci. Tobramycin was more active in clinical *Staphylococcus*. The mec A gene was present in community *S.aureus*, clinical SCN and clinical *S. aureus* strains. The amplified gene fragments were of the same molecular weight. It is likely that he has a dissemination of clinical strains in the city as well as resistance genes. All strains carrying the mec A gene were resistant to macrolides alone and / or related. The risk of dissemination and therapeutic impasse linked to these generally multidrug-resistant strains requires the adoption of control measures to reduce and prevent their emergence. The measures to be implemented include the correct use of antibiotics, strict compliance with hygiene rules, the geographical and technical isolation of patients, epidemiological surveillance and policies to fight nosocomial infections.

A data availability statement is compulsory for research articles and clinical trials. Here, authors must describe how readers can access the data underlying the findings of the study, giving links to online repositories and providing deposition codes where applicable.

Conflicts of Interest

"The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper."

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