RESEARCH ARTICLE

ANTIMICROBIAL RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATES FROM INPATIENTS AND OUTPATIENTS IN OUAGADOUGOU, BURKINA FASO

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Abstract

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Staphylococcus aureus, which is described as a common nosocomial pathogen has acquired an ability to cause a wide range of infections and has been found to be one of major organisms associated with multi-drug resistant and high morbidity and mortality worldwide. The objective of this study was to assess the antibiotic resistance of Staphylococcus aureus strains isolated from clinical specimens in Ouagadougou. *Staphylococcus aureus* strains were isolated from different clinical specimens collected in four hospitals in Ouagadougou for a period running from April 2018 to March 2019. Classical bacteriological methods were used for isolation and identification of the strains. All strains were subjected to a set of 12 antibiotics to study their antibiogram by using the Kirby-Bauer disk diffusion method. A total of 150 isolates of S. aureus were collected during the study period. S. aureus isolates were mostly from pus specimens (60%) from patients between 20 and 59 years old. Antibiotics resistance testing showed that the proportion of methicillin-resistant *Staphylococcus aureus* (MRSA) among the isolates was 31.33% (47/150). Most of these MRSA (63.83%) were multidrug-resistant. 71.43% of MRSA isolates exhibited resistance to several antibiotics, while 42.4% of MRSA isolated in outpatient was multidrug-resistant strains. Tetracyclin resistance was very frequent (58.7%), followed by levofloxacin (56.25%), ciprofloxacin (44.12%), kanamycin (36%) and erythromycin (30%). This multidrug-resistance was most frequent in isolates from hospitalized patients. This study has revealed alarming levels of MRSA isolates and high cross-resistance to antibiotics other than those of the free-lactam family in Ouagadougou.

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Introduction:-
Antimicrobial resistance (AMR) is an increasingly serious threat to global public health (WHO, 2018). The global health, the food security and the development of humanity are thus threatened. Indeed, this AMR leads to higher

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medical costs, prolonged hospital stays, and increased morbidity and mortality (Founou et al., 2017)**.** This antimicrobial resistance is putting the gains of the Millennium Development Goals at risk and endangers achievement of the Sustainable Development Goals and requires action across all (WHO, 2018). *Staphylococcus aureus*, a bacterium commonly found on the skin and in the nose of about 30% of individuals has become one of the major disease-causing organisms because of AMR. The infections list ranges from benign cutaneous lesions (furuncles, paronychia) to fatal sepsis and toxic shock syndrome (Tong et al., 2015; Hale et al., 2018). Staphylococcus aureus has been responsible for almost 17.5% of sepsis in children in hospitals (Odutola et al., 2019). The emergency of Methicillin-resistant *Staphylococcus aureus* (MRSA) strains or multidrug-resistant *S. aureus* strains, in the last decade has resulted rapidly progressive, potential fatal diseases including life-threatening pneumonia, necrotizing fasciitis, endocarditis, osteomyelitis, severe sepsis, and toxinoses (Monecke et al., 2011). MRSA is associated with significant mortality and morbidity and imposes a serious economic burden on scarce healthcare resources worldwide. According to the World Health Organization (WHO), methicillin-resistant *Staphylococcus aureus* increases the death rate of infected patients by 64%. The use of different types of antibiotics over the years has led to the emerging of multi-resistant *Staphylococcus aureus* strains. The MRSA prevalence varies according to the regions and the living conditions of the populations (Schaumburg et al., 2015). The frequency of MRSA in West Africa, is generally high: 20-43% in Nigeria (Ghebremedhin et al., 2009, Akerele et al., 2014), 36% in Benin (Ahoyo et al., 2006) and 35.7% in Togo (Kombate et al., 2011). In Burkina Faso, there are few studies on methicillin-resistant *Staphylococcus aureus* (MRSA) strains and their antibiotics susceptibility. This study was carried out to determine the prevalence and antibiotics susceptibility profile of MRSA isolated from pathological samples in different medical centres in the city of Ouagadougou, Burkina Faso.

Material and Methods:-
Sampling:
Clinical strains have been collected in different health facilities in Ouagadougou (Burkina Faso), between April 2018 and March 2019. *Staphylococcus aureus* isolates were isolated from different clinical samples (pus samples, vaginal swabs, and urine and blood culture samples) from different medical microbiology laboratories.

Microbiological analyses:
S. aureus strains have been identified based on morphological characteristics and ability to ferment mannitol to Chapman mannitol media. The colonies of golden yellow that fermented mannitol were subjected to Gram staining and biochemical tests. Biochemical tests used to confirm S. aureus strains included tests of catalase, coagulase, and Dnase. Subsequently, the DNase agar plates were flooded with HCl (1N) (Oxoid Limited, UK). Isolates that showed the ability to hydrolyze DNA were identified as *S. aureus*.

Antimicrobial susceptibility testing:
Antibiotics susceptibility testing was carried out on Mueller Hinton Agar (MHA) (HIMEDIA, India, REFM1084-500G) using the Kirby-Bauer disc diffusion technique. Screening of MRSA was done using cefoxitin disc (30µg) according to the Clinical and Laboratory Standards Institute (CLSI) recommended disc diffusion method. A reference strain of *Staphylococcus aureus* (ATCC 25923) was used as a control strain. The antibiotics discs used for identification of antibiotic sensitivity pattern of MRSA isolates were: erythromycin (15 µg), tetracycline (30 g), gentamicin (10 µg), clindamycin (2 µg), ciprofloxacin (5 µg), levofloxacin (30 µg), tobramycin (30 µg), kanamycin (30 µg), cotrimoxazole (1.25 + 23.75 µg), fusidic acid (10 µg), chloramphenicol (30µg) (HIMEDIA, India, REFM1084-500G). Each test isolate was emulsified in peptone water to create a suspension with turbidity similar to 0.5 McFarland standards. The swab was dipped into the suspension and pressed against the interior walls of the container to drain excess fluid. The dried surface of a MHA plate was inoculated by streaked the swab over the entire agar surface. After an application of antibiotic discs to inoculated agar plates, the plates were inverted and incubated at 37^oC for 18–24 h. The diameter of the zones of inhibitions around the antimicrobial discs were measured and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST/CA-SFM, 2018).

The multi-resistant (MRSA) is defined as the resistance to at least of three different antibiotics family.

Statistical analysis:
The data obtained were analysed with SPSS software version 21 using simple descriptive statistics. Tests for significance for associations computed for categorical variables were based on the chi–square. P-values less than 0.05 ($p < 0.05$) were regarded as statistically significant.

Results:-
Bacterial Strains:
A total of 150 isolates were collected during the study period (103 strains were from outpatients and 47 from hospitalized patients over 2 days). Most of isolates were isolated in adults' patients (20-59 years' group) (Table 1). *Staphylococcus aureus* strains have been isolated from different pathological products as shown in Table 2. It can be noticed that most of these strains were from pus samples (90/150), followed by those obtained from urine samples (30/150) and from vaginal swabs (24/150). Only six (6) S. aureus strains were from blood specimens.

Table 1:- S. aureus Methicillin-resistant association with age.

Age group	MRSA (N(%))	MSSA (N(%))	Total(N)	P value
≤20	10 (21.3)	27 (26.2)	37	0.423
21-59	28 (59.6)	62 (60.2)	90	0.005
≥60	9 (19.1)	14 (13.6)	22	1.099
Total	47	103	150	

Table2:- *S. aureus* Methicillin-resistant association with different types of clinical specimens.

Specimen type	MRSA (N(%))	MSSA (N(%))	Total(N)	P value
Pus	26(55.3)	64(62.1)	90	0.625
Urine	10(21.3)	20(19.4)	30	0.070
Vaginal swab	8(17)	16(15.5)	24	0.053
Blood culture	3 (6.4)	3(2.9)	6	1.012
Total	47	103	150	

Antimicrobial susceptibility testing:
Out of 150 S. aureus collected in Ouagadougou, 47 (31.33%) were *Staphylococcus aureus* Methicillin-Resistant (SAMR).The distribution of MRSA by age of patients was significantly high in adults (20-59 years' group) ($p < 0.05$). Distribution of the frequency of MRSA isolates according to outpatients and inpatients (Table 3) showed that the high percentage of isolates was 70.2% from outpatients. It was no significant difference ($p > 0.05$) between numbers of isolation in different hospital ward.

Table3:- *S. aureus* Methicillin-resistant association with in outpatients and inpatients.

Characteristic	MRSA (N(%))	MSSA (N(%))	S. aureus (N)	P value
Outpatients	33(70.2)	70(68)	103	
Inpatients	14(29.8)	33(32)	47	0.558

The antibiotic sensitivity pattern of MRSA isolates are given in figure 1. *Staphylococcus aureus* strains were resistant to 5 antibiotics. A higher frequency of antimicrobial resistance was observed to tetracycline (58.7%), followed by levofloxacin (56.25%), ciprofloxacin (44.12%), kanamycin (36%) and erythromycin with (30%).

Most of the strains (61.7%) of the MRSA were multidrug-resistant (Table 4). Seventy-one percent (71.43%) of MRSA isolated in inpatient had resistance to several antibiotics, compared to MRSA strains isolated from outpatient (42.4%) (Table 5). Most MRSA that had multi-antibiotic resistance were isolated from patients who had spent more than two days in hospital care.
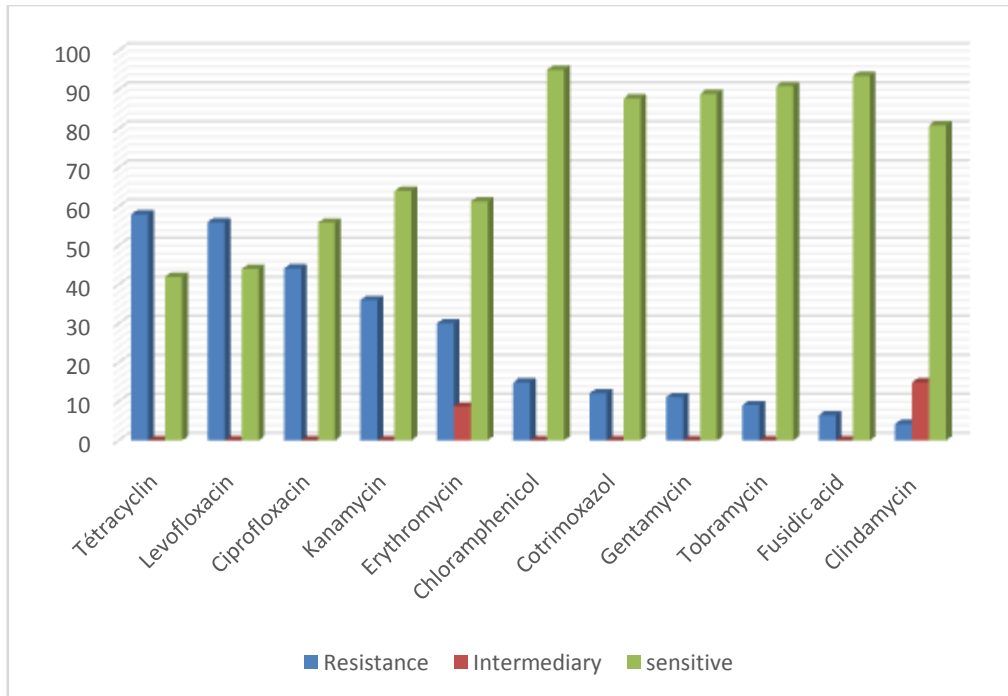
Figure1:- The antibiotic sensitivity pattern of MRSA.

Table 4:- Resistance of MRSA strains to antibiotics.

Antimicrobial	Number of resistant MRSA (N)	Resistant (%)
Da +K + Tob+ Gen + Té+ P	1*	2
C +P	1	2
Cip+ P	1	2
E P	1	2
E + Cip + P	2*	4
E + Té + P	1*	2
E + Té +Cip + Cot + P	1*	2
E + Té + Cot + P	1*	2
Fc + Cot + P	1*	2
E + Gen + P	1*	2
E + K + Tob + Gen + Cip +P	1*	2
Té + Le + Da + P	1*	2
Gen + Fc+ P	1*	2
Tob + Gen +Té +P	1*	2
K + Cip + P	1*	2
E + K + Cip + Lev + P	3*	6
E + K + C + Lev + P	1*	2
E + K + Té + Cip + Lev + P	1*	2
E + K + Té + Cot + P	1*	2
Té + P	9	18
Té + C + P	5*	10
Té + C + Cot + P	1*	2
Té + Cip + P	2*	4
Té + Cip + Lev + P	2*	4

C: chloramphénicol; Cip: ciprofloxacin; E: erythromycin; Te: tetracycline; Cot: trimethoprime/sulfamexazol ; Fc: fusidic acid; Gen: gentamycin; Lev: levofloxacin; (*): Multi-drug resistance of strains. P: penicillin, K: kanamycin, Tob: tombramycin, Da: clindamycin

Table5:- Multidrug resistant isolates among Outpatient and Inpatient.

characteristic	MRSA/MDR (N(%))	MRSA/NoMDR (N(%))	MRSA (N)
Outpatient	14(42.4)	19(57.6)	33
Inpatient	4(28.57)	10(71.43)	14

P > 0.05. MRSA: Methicillin resistant Staphylococcus aureus, MDR: Multidrug resistance

Discussion:-

Staphylococcus aureus is mainly present in pus samples. In this study, a majority of isolates were from pus specimen (60%). A similar isolation rate (64.8%) of Staphylococcus aureus in pus samples was reported by Senthamarai et al. (2019). Indeed, S. aureus skin infections are accompanied by production of pus resulting from the proliferation of bacteria. Thus, Sawadogo et al. (2019), Sarwar et al. (2014) found that Staphylococcus aureus was the most frequently isolated germ in pus samples (37.8% and 64%, respectively). Other *Staphylococcus aureus* strains isolated from urine samples (20%), vaginal swabs (16%) and blood culture samples (4%). His involvement in urinary, vaginal and blood infections was demonstrated by the authors as Kengne et al. (2019), Garoy et al. (2019), Sina et al. (2018).

Out of 150 *Staphylococcus aureus* strains collected in Ouagadougou, forty-seven (31. 33%) were MRSA. This study confirms the preponderance of *Staphylococcus aureus* in community and hospital infections and documents the high frequency of MRSA during infections in the city of Ouagadougou. Similar results have been observed by Senthamarai et al., (2019), Abdullahi and Iregbu (2018), Rashmi et al. (2017), Jaydev et Mitesh (2017), Kombaté et al. (2011), Ahoyo et al. (2006), who had recorded prevalence rates of 21.87%, 26.9%, 29.89%, 35.56%, 35.7%, 36% respectively. Gupta et al. (2017), Kengne et al. (2019), Garoy et al. (2019) reported a higher rate of 76.75%, 80.4%, 72% respectively. Prevalence variations observed with different studies could be explained by different variations in sample size, specimens collected, and duration of study, populations studied and methods used for detection.

The 19–50 age group had significantly the highest rate of MRSA isolation with 28(59.6%). The majority of the strains collected in this study were from patients in this age group. Garoy et al. (2019) reported a significantly higher in patients below 18 years and significantly lower in patients above 61 years old. These results show that age may be a risk factor for MRSA infections.

Distribution of the frequency of MRSA isolates according to the hospital ward showed that the high percentage of isolates was from outpatients (70.2%). The study shows the preponderance of MRSA in community infections. Community MRSA had already been documented byAkpaka et al. (2015), Kombaté et al. (2011), Abdullahi and Iregbu (2018).

More than 30% of MRSA isolates were resistant to tetracycline (58.7%), followed by levofloxacin (56.25%), ciprofloxacin (44.12%), kanamycin (36%) and erythromycin (30%). The high resistance of MRSA isolates to antibiotics could be due to the widespread use of these antibiotics for the treatment of staphylococcal infections in healthcare, because high consumption of antibiotics leads to the emergence of antibiotic-resistant Staphylococcus aureus. Multidrug resistance to these old and commonly used antimicrobials is a public health problem and could facilitate usage of fluoroquinolones and third-generation cephalosporins for empiric treatment (Somda et al., 2017).

Resistance of MRSA to tetracycline was found at 58% in the study, which is correlated with previous findings that MRSA isolates were 53.2% and 42% (Rajesh et al., 2018; Siddiqui et al., 2017). However, another study found 89.4% resistance to tetracycline (Osiyemi et al., 2018). This study reported 44.12% resistance to ciprofloxacin by MRSA isolates, and other studies had 23.8%, 47%, 74.8% resistance to ciprofloxacin (Bindu et al., 2017; Siddiqui et al., 2017; Rajesh et al., 2018). It was documented 30% resistance to erythromycin in MRSA isolates. Rashmi et al. (2017) have showed that all MRSA isolates in their study showed resistance to erythromycin.In our study, it was noted the emergence of strains resistant to ciprofloxacin, levofloxacin and kanamycin. This situation might be explained by the fact that in developing countries, populations frequently practice self-medication and use proscribed drugs sold on the street because of their low economic power and their ignorance of the danger of these practices. These practices increase the phenomenon of bacteria resistance (Somda et al., 2017). These observations suggest the necessity to undertake the regulation of antibiotics usage in Burkina Faso to avoid or control the spread of resistance to antimicrobials.

The high frequency of multiple resistance of MRSA to antibiotics in hospital care units could be related to inappropriate use of antibiotics, self-medication, and practices for preventing and controlling non-standard infections.

Conclusion:-
This study documents the strong involvement of MRSA in community and hospital infections in Ouagadougou. Among the risk factors associated with the acquisition of MRSA are the older age, prolonged hospitalization, previous antibiotics. The increase in the number of methicillin-resistant strains and their multiresistance to antibiotics is a problem in the management of bacterial infections. Establishing measures to control antibiotic infections and management could help reduce the emergence and spread of these MRSA.

Conflicts of interest:
The authors declare that they have no competing interest.

Authors' contributions:
TR carried out the sampling and strains isolation and their antibiotics susceptibility and drafted the manuscript; ZC, KDS, SNS, TY, LS, SA and TY supervised the sampling and strains isolation, antibiotics susceptibility and participated in writing the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgement:-

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