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ANTIBIOTIC RESISTANT PROFILE OF COAGULASE POSITIVE *STAPHYLOCOCCUS* SPECIES AMONG CLINICAL ISOLATES IN ZARIA METROPOLIS, KADUNA

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ABSTRACT

Coagulase positive *Staphylococci* (CoPS) are usually pathogenic causing diseases ranging from mild to severe. This study was to determine the occurrence and antimicrobial resistant profile of coagulase positive *Staphylococci* (CoPS) from different clinical source. A total of 150 presumptive coagulase positive *staphylococcal* isolates from all specimens, submitted to the laboratory microbiology unit of the hospitals were collected, and screened using Microbiology standards. Disc agar diffusion method was used for eleven (11) antibiotics susceptibility test while vancomycin screening agar and Broth Micro dilution test was used for vancomycin susceptibility evaluation. Biofilm assessment was performed using congo red agar (CRA) and Microtitre plate assay method. Percentage Occurrence of coagulase positive reveals *S aureus (21%), S intermedius (15%), S pseudintermedius (7%), S hyicus (27%), S schleiferi (4%), S lutrae (14%).* Antibiotic susceptibility testing revealed resistance pattern ranging from 18 to 78%. With Non MDR 14(19%), MDR 44(60%), XDR 13 (18%) and PDR 2(18%). Phenotypic evaluations showed 75% strains to be *MRCoPS*, 37% as *VRCoPS*, and 22% as both *MRCoPS* and VR*CoPS.* Biofilm strains revealed 66% and 75% of isolates using CRA and Microtitre plate assay respectively. The emergence of MDR has resulted to ineffective therapy. An urgent therapeutic strategy is needed in our clinical settings to stem the tide of difficult to treat infections.

Keywords*: Coagulase Positive Staph* Spp*,* Multidrug, Extended Drug, Pan Drug Resistance

INTRODUCTION

Coagulase positive *Staphylococci* (CoPS) are usually pathogenic and can cause diseases ranging from mild to severe. Special attention has been focused on Methicillin and Vancomycin Resistant *Staph aureus*, but other CoPS can also represent an important threat (Morar *et al*., 2021). CoPS is also considered a leading cause of several health-care-associated infections (e.g. skin and soft tissue abscesses, endocarditis, osteomyelitis, bacteremia) (Todar, 2020). The emergence of MDR in hospital-acquired (HA) infections highlights this species as a potential pathogen that is able to cope with the antimicrobial agent and also in different patient populations is a major public health concern. (David *et al.,* 2021)

The research was to determine the occurrence of coagulase positive *Staphylococci* (CoPS) and to assess their antimicrobial resistant profile from different clinical source so as to provide necessary information for accurate therapeutic managements of infections caused by such organism (WHO,2020).

METHODS

Study Area

This study was carried out using two selected hospitals within Zaria metropolis. ABUTH Shika and ABU Medical Centre Main campus Zaria, Kaduna State.

Ethical approval

Ethical clearance was collected from the Health Research Ethics Committee, ABUTH Shika and Committee on Use of Human Subject for Research, ABU Medical Centre, Samaru Zaria.

Collection of clinical Isolates

A total of 150 suspected non duplicate coagulase positive *staphylococcal* isolates from all specimens (blood, urine, high vaginal swab, wound swab, ear swab, endocervical swab) submitted to the laboratory microbiology unit of the selected hospitals were collected over a period of 6 months and transported in a sterile Nutrient agar slant ice park to Pharmaceutical Microbiology Lab A.B.U. Zaria.

Identification of Coagulase Positive *Staph* spp isolates

Microbiology standard procedures ((tube coagulase test, growth on mannitol salt agar & Dnase agar) Microgen *Staph* identifcation kit (bioMerieux, Inc, Durham, USA) was used to identify the *Staph spp* isolates. The procedure was carried out according to the manufacturer's instructions.

Antibiotic susceptibility test; Kirby–Bauer Disk diffusion tests was performed for each of the isolates previously identified following the method recommended by the Clinical Laboratory Standard Institute (CLSI, 2020). A disk containing the following antibiotics, gentamicin (10µg), ceftaroline (30µg), trimethoprim/sulphamethoxazole (1:19) (25µg), ciprofloxacin (5µg), chloramphenicol (30µg), cefoxitin (30µg), erythromycin (30µg), nitrofurantoin (30µg), clindamycin (30µg), vancomycin (30µg), doxycycline (30µg), linezolide (30µg) (Oxoid Ltd. Basingstoke, London) was used.

Determination of vancomycin susceptibility

The vancomycin minimum inhibitory concentration (MIC) of *S. aureus* was determined based on 0.5 McFarland standards by using Micro dilution Broth and Vancomycin screening agar 6µg/ml BHI (Brain Heart Infusion agar) CLSI 2020. Visible growth was recorded after 24 hours incubation at 37°C in each concentration.

Assessment of biofilm formation using Congo red agar (CRA) Based Method

The agar medium to be used was prepared by adding 37 g of the Brain Heart Infusion (BHI) powder, 50 g of sucrose and 10 g of agar in 1 L of distilled water. The mixture was then autoclaved for 15 min at 121° C. The agar solution was cooled down to about 50° C and a solution of Congo red (8 g/L) was added and mixed. The media was poured into the Petri dishes and allowed to solidify. The solid plates were inoculated with the microorganisms and incubated at 37° C for 24 h. The plates were read the next day and the organisms considered positive (biofilm-producers) if black colonies were observed on the Congo red agar and negative (non-biofilm producers) if pink, or red-orange colonies were observed on the Congo red agar (Idrees *et al*., 2021).

Microtitre plate assay method

Three wells of sterile 96-microtiter polyester U-bottomed plate were filled with 200 µl of bacterial suspension (dilution 1:100 with fresh medium). After 15 min, plates were stained for 5 min with 0.2 ml of 2% crystal violet per well. Excess stain was removed and rinsed off by placing the plates under running tap water. The plate was air-dried. (Christensen *et al*., 1985). The optical

density (OD) of each was measured at 570 nm. The adherence ability of tested isolates was classified into four categories based on the obtained OD as strongly adherent (OD570 ≥ 3.0) +++, moderately adherent (OD570 ≥ 1.5–2.0) ++, weakly adherent (OD570 ≤ 0.5– 1.0) +, and nonadherent (OD570 < 0.5) -, (OD570 of negative control). (Alcaráz, Satorres, Lucero, & Centorbi, 2003).

Statistical analysis

Results were recorded as averages and percentages.

RESULTS

Rate of Occurrence of Coagulase Positive *Staph spp* collected from the hospitals

A total of 150 isolates collected from two Hospitals in Zaria were analysed for CoP *Staph* spp. as shown in Table 1.

Distribution of Coagulase Positive *Staph spp* Isolates by Source

The result for distribution of Coagulase Positive *Staph* spp isolates by source as shown in Table II.

Distribution of Coagulase Positive *Staph* Spp Isolates by Age and Gender

It was observed that the isolates were mostly isolated from female than male from age range >31 years as shown Table III.

Table I: Rate of occurrence of Coagulase Positive *Staph* spp collected from the hospitals

Hospital	No. of Isolates screened	No of CoP *Staph* isolated	% Prevalence
ABUTH	130	68	52
ABUMEC	20	5	25
Total	150	73	49

Table II: Source Distribution of Coagulase Positive *Staph* Spp Sample

S/N	SAMPLE SOURCE	ABUTH N (%)	ABUMEC N (%)	TOTAL N (%)
1	Blood	10 (15)	0(0)	10 (14)
2	Boil	2 (3)	0 (0)	2 (3)
3	Ear	3 (4)	0 (0)	3 (4)
4	ECS	1 (2)	0 (0)	1 (2)
5	HVS	3 (4)	1 (20)	4 (5)
6	Urine	6 (9)	4 (80)	10 (14)
7	Wound	43 (63)	0 (0)	43 (59)
	TOTAL	68 (100)	5 (100)	73 (100)

Table III*;* Age and Gender Distribution of Coagulase Positive *Staph* Spp

S/NO	AGE RANGE	No (%)	MALE n (%)	FEMALE n (%)
1	≤ 5 YEARS	6 (8)	2 (2)	4 (6)
2	6 – 15 YEARS	7 (10)	1 (1)	6 (9)
3	16 – 30 YEARS	22 (30)	13 (18)	9 (12)
4	≥ 31 YEARS	38 (52)	20 (27)	18 (25)
	TOTAL	73	36 (49)	37 (51)

Occurrence of coagulase positive *staph* spp

It was observed that *Staph hyicus* was mostly isolated than other CoP *Staph* Spp as shown in Table IV.

Antibiotics resistant profile of Coagulase Positive *Staph* Spp isolates

Antibiotic testing revealed resistance pattern ranging from 18 to 78% (Table V).

Table IV: Occurrence of coagulase positive *staph* spp

S/NO	*Staph* spp	No	%
1	*Staph aureus*	15	21
2	*Staph hyicus*	20	27
3	*Staph intermedius*	11	15
4	*Staph pseud intermedius*	5	7
5	*Staph lutrae*	10	14
6	*Staph schleiferi*	3	4

Table V: Antibiotics Resistant Profile of Coagulase Positive *Staph* Spp

S/No	ANTIBIOTICS	I (%)	R (%)	TOTAL (%)
		n (%)	n (%)	n (%)
1	Cefoxitin	0 (0)	55 (75)	55 (75)
2	Chloramphenicol	7 (10)	25 (34)	32 (44)
3	Ceftaroline	11 (15)	25 (34)	36 (49)
4	Ciprofloxacine	12 (16)	42 (58)	54 (74)
5	Clindamycin	4 (6)	31 (42)	35 (48)
6	Erythromycin	7 (10)	38 (52)	45 (62)
7	Doxycycline	3 (4)	54 (74)	57 (78)
8	Gentamicin	8 (11)	32 (44)	40 (55)
9	Linezolide	0 (0)	13 (18)	13 (18)
10	Nitrofurantoin	12 (16)	13 (18)	25 (34)
11	Vancomycin	12 (16)	15 (21)	27 (37)
12	Sulphamethoxazole trimethoprim	1 (2)	47 (64)	48 (66)

Key; I- intermediate resistance, R- resistant

Biofilm Forming Amongst Coagulase Positive *Staph* Spp Isolate

It was observed that 75% of the isolates were biofilm formers with Micro titre plate assay method, and 66% positive with Congo Red agar as shown in Table IV.

Degree of Resistance Coagulase Positive *Staph* Spp

The degree of resistance was classified as Non MDR, XDR and PDR as shown in chart below.

Table VI: Biofilm forming amongst coagulase positive *staph* spp isolate

Method	Weak	Moderate	Strong	Total n (%)
Congo Red	8	0	40	48 (66)
Microtitre plate assay	13	18	24	55 (75)

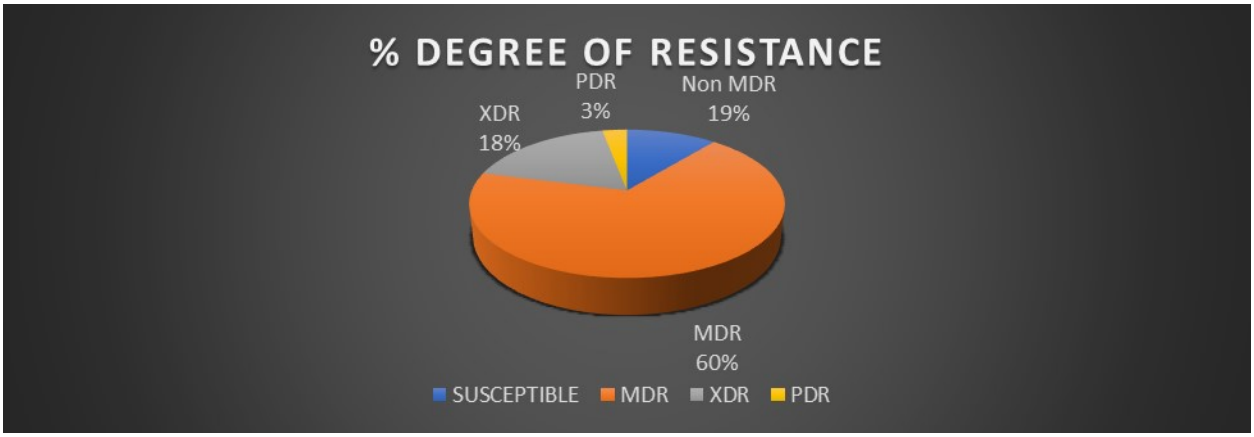

Figure I: Degree of Resistance Coagulase Positive *Staphylococcus* Spp

Key; MDR- Multidrug resistant, XDR- Extended drug resistant, PDR- Pan drug resistant

DISCUSSION

Coagulase positive *Staphylococci* (CoPS) are usually pathogenic causing diseases ranging from mild to severe. It was observed in these facilities that there is possibility of inappropriate diagnosis of CoP *Staph spp*. Which has been considered *Staph aureus* in diagnostic reports. Meanwhile other CoP *Staph spp* exist in clinical specimens which are barely recognized (Morar *et al*., 2021). In this study, the prevalence of CoP *Staph spp* was 49% and 21% were *Staph aureus*, majorly from wound (SSI), mostly from females with 78% MDR to antibiotics used in management of *Staph* spp. Joshua *et al*., 2022, reported similar findings with 20.5% *Staph aureus*, majorly from wound (SSI), mostly from females with 90.5% MDR. The reason bounces back to records of females' visits exceeds that of males in the hospitals.

Infections of wounds are re-emerging medical problem worldwide, especially because of multidrug resistance nature of their causative organism, with consequent

huge economic burden, morbidity and mortality (Janssen *et al*., 2018). In Zaria, high percentage resistance was observed with penicillin's 100% and most susceptible to gentamicin 96.1% (Joshua *et al*., 2022), which is similar to the results of Garba *et al*.,2018 and also the results in this study, with 78% resistance to penicillins but most susceptible to linezolide, nitrofurantoin and vancomycin, aside gentamicin, which are used in management of infections caused by CoPS and are not commonly used in this locality.The High antibiotic resistance, suggests that, the isolates were originated from an environment where antibiotics are frequently used and the rate of resistant strains occurrence is associated to many factors which includes, lack of infection prevention which contributes to recurrence of infection, misuse of antimicrobials from prescription–dispensing-to patient use (Deyno *et al.,* 2017), antibiotics purchased without prescription, which leads to misuse of antibiotics by the public, by health professionals and non-standardized practice (Ayukekbong *et al*., 2017). Another factor could be due to poor hospital hygienic conditions. Lack of culture and routine antimicrobial susceptibility testing which diverts to empiric therapy (Deyno *et al.,* 2017)

Moreover, antimicrobial resistance could be associated with several virulent factors such as biofilm. Indirect observation of biofilm using Microtitre plate assay method revealed biofilm (75%) in these isolates. The biofilm formation ability of this organism complicates effective therapeutic management of infections caused by the organism, thus the leading organism in 80% major health problems due to biofilm-associated infection (Fany *et al*., 2017).

In line with strategies for prevention and containment of infections caused by CoP *Staph* spp*,* there is a need for innovative way of halting AMR. Combination therapy, design of new drugs and availability of alternative antimicrobial agents will play important role in fighting against AMR.

CONCLUSION

The results in this study have exposed the occurrence of Coagulase Positive *Staph* Species with *Staph. hyicus,* the most prevalent. High multidrug resistance has been observed which poses a therapeutic challenge in treatment of infections caused by this organism in this locality. However, Linezolid, Nitrofurantoin, and Vancomycin were observed to be the most effective against the strains.

Conflict of Interest: The authors declare that they have no conflict of interest.

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