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Short Communication

Prevalence of methicillin resistant *Staphylococcus aureus* and resistance pattern of its clinical strains to beta-lactam antibiotics

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ABSTRACT

Staphylococcus aureus is the leading overall cause otf nosocomial infections with increasing resistance to β lactam antibiotics. This study was carried out to study the current resistant/susceptibility pattern of *S. aureus* to β lactam antibiotics and prevalence of Methicillin Resistant *S. aureus* (MRSA) in the studied population. Clinical isolates of *S. aureus* strains were collected from Medical Microbiology Unit of University College Hospital, Ibadan between May and October, 2012. The isolates were confirmed through growth on Mannitol Salt Agar (MSA) and tube coagulase test. The susceptibility / resistance pattern of the *S. aureus* strains to antibiotics were tested by disc diffusion method. Fifty studied *S. aureus* strains were highly resistant to Amoxycillin (92%), Aztreonam (70%), but high susceptibility was observed to Imipenem (90%), Cefotaxime (62%), Ceftazidine (50%), Cefoxitin (66%), Ceftriazone (52%), Amoxycillin/Clavulanic acid (50%), *S. aureus* strains (42%) that were resistant to amoxicillin were susceptible to amoxicillin/clavulanic acid while 34% of the studied S. *aureus* strains were MRSA. The relatively high prevalence of MRSA in the studied *S. aureus* strains call for surveillance studies and implementation policies in control of MRSA. Cephalosporins are still relatively effective for treatment of *S. aureus* infections. The observed synergy in this study between imipenem and aztreonam is an indication that combine therapy of imipenem and aztreonam will lead to enhanced antimicrobial activity of aztreonam.

Key words: *Antibiotics, Resistance, S. aureus, MRSA*

INTRODUCTION

Staphylococcus aureus has remained a major human pathogen; it is estimated that 20% of the human population are long-term carriers of *S. aureus* (Klytmans *et al.*, 1997). *S. aureus* colonizes healthy individuals and causes severe infection in hospitalized patients (Brook *et al.*, 2004). It is responsible for causing a variety of human infections, which may range from minor skin diseases to life-threatening infections. *S. aureus* isolates worldwide are increasingly resistant to a greater number of antimicrobial agents; inevitably this has left fewer effective bactericidal antibiotics to treat these often life-threatening infections. As rapidly as new antibiotics are introduced, staphylococci have developed efficient mechanisms to neutralize them.

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Historically, β-lactam antibiotics have exhibited potent activity against *S. aureus*, which along with good safety profiles make them the agents of choice for the treatment of staphyloccocal infections. Penicillin, the first class of the β-lactam antibiotics discovered, gave rise to successive groups of related β-lactams for the treatment of bacterial infections. With the development of resistance, however, many of these β-lactams are ineffective against a significant proportion of *S. aureus* clinical strains (Guignard *et al.*, 2005; Llarrull *et al.*, 2009). There are currently overwhelming resistance by *S. aureus* to almost all classes of β-lactam antibiotics with a growing threat in the treatment of *S. aureus* infections due to the rise of resistance to conventional antibiotic treatments (Diekema, *et al.*, 2001).

An initial response to penicillin resistance was the development of methicillin (Bennett *et al.*, 1999) but, a serious concern has arisen from the increase in infections by strains of *S. aureus* called Methicillin-Resistant-*Staphylococcus aureus* (MRSA) (Talaro, 2008). MRSA is a serious threat to hospitalized patients globally as well as to the community at large (Onanuga *et al.*, 2005). MRSA strain is of concern not only because of its resistance to methicillin but also because of its general resistance to many other chemotherapeutic agents (Vidhani *et al.*, 2001). Resistance in MRSA is related to a chromosomal mecA gene that specifies the production of an abnormal penicillin binding protein called PBP2a or PBP21.

Penicillin–binding proteins are targets for beta-lactam antibiotics. PBP2a has a decreased affinity for binding beta-lactam antibiotics resulting in resistance not only to methicillin but also to all beta-lactams including penicillins and cephalosporins (Weems, 2001; Onanuga *et al.*, 2005).The emergence of MRSA has posed a serious therapeutic challenge in the treatment of *S. aureus* infections by ß-lactam and related group of antibiotics. Moreover, the overall incidence of MRSA isolation is gradually increasing. Therefore investigation, documentation and understanding of the prevalence of MRSA infections in different countries are of significant importance. This controls indiscriminate and irrational use of antibiotics and it is useful for implementations of measures to control the spread of MRSA in hospitals and community. The objectives of this study therefore are to estimate the prevalence of MRSA from clinical samples isolated from University College Hospital (UCH), Ibadan, Oyo State, Nigeria, to observe the resistance pattern of *Staphylococcus aureus* to beta-lactam antibiotics, and also observe if any β lactam antibiotics is still effective for the treatment of *S. aureus* and MRSA infections.

MATERIALS AND METHODS

Bacterial Isolates

Microbial isolates were collected between May and October 2012 from Medical Microbiology Department, University College Hospital, Ibadan, Oyo State, Nigeria. They were isolated from clinical samples of wound swab, blood culture, nasal swab, eye swab and ear swab. All collected isolates were streaked on mannitol salt agar (MSA) (Oxoid, UK) and incubated at 37ºC for 48 hours. All isolates that grew on MSA with characteristic yellow colonies were subjected to a confirmatory coagulase test by inoculating overnight cultures into 1ml of fresh human plasma dispensed into sample bottles. This was incubated for 4 to 24 hours after which the plasma was observed for clot formation.

Susceptibility testing of Staphylococcus aureus strains

The susceptibility of the *S. aureus* strains to different antibiotics was tested according to Clinical Laboratory Standard Institute (CLSI) performance standards for antimicrobial disc diffusion and agar dilution susceptibility tests and WHO requirements (CLIS, 2010) using standard antibiotic discs (Oxoid, UK). A 18-hr broth culture of confirmed *S. aureus* strains of approximately 10^5 cfu/ml which is equivalents to MacFarland standard 1 was inoculated onto Muller Hinton agar by spread plate method. Ten different standard antibiotic disks (Oxoid, UK) -Amoxycillin (AML), Erythromycin (E 15 µg), Ceftazidine (CAZ 30 µg), Clindamycin (DA 2 µg), Cefotaxime (CTX 30 µg), Imipenem (IPM 10 µg), Aztreonam (ATM 30 µg), Ceftriaxone (CRO 30 µg), Amoxycillin/Clavulanic acid (AMC 20/10 µg) and Cefoxitin (FOX 30 µg) were placed firmly at least 2 cm apart on the agar plates using a sterile forceps. The plates were kept on the bench for 30 min to allow diffusion of the antimicrobials before incubating at 37ºC for 24 hrs. The plates were examined for clear zones of inhibition around the discs. The diameter (mm) of zone of inhibition was measured and compared with standard zones to determine resistance/susceptibility

RESULTS

Fifty *S. aureus* strains that grew on MSA with yellow colonies and also coagulated plasma were tested for their antibiotic resistance/susceptibility pattern using standard discs. All the isolates were resistant to ≥2 antibiotics. The isolates were relatively susceptible to

cephalosporins and imipenem while generally resistant to other tested β-lactams antibiotics. The *S. aureus* strains were highly resistant to Amoxycillin (92%), Aztreonam (70%), Erythromycin (72%) and Clindamycin (78%). But high susceptibility was observed to Imipenem (90%), Cefotaxime (62%), Ceftazidine (50%), Cefoxitin (66%), Ceftriazone (52%), Amoxycillin/Clavulanic acid (50%) (Table I). Forty-two percentage (42%) of the *S. aureus* strains that were resistant to amoxicillin were susceptible to amoxicillin/clavulanic acid. All the isolates resistant to amoxicillin/clavulanic were susceptible to ≥ 1 of the cephalosporins. 34% of the studied *S. aureus* strains were MRSA with observed resistance to cefoxitin. All the MRSA were resistant to amoxicillin, amoxicillin/clavulanic acid and ≥1 cephalosporin except *S. aureus* DF032 which was sensitive to all tested cephalosporins. The tested MRSA were also resistant to erythromycin and clindamycin except *S. aureus* DF038 which was sensitive to clindamycin however, 88% of the studied MRSA strains were sensitive to imipenem. (Table II). An interesting synergy was observed in isolates *S. aureus* DF44 and *S. aureus* DF46 which were resistant to aztreonam but sensitive to imipenem with an observed synergy in between the two antibiotics. (Fig. I)

Table 1:
Resistant/Susceptibility pattern of *S. aureus* strains to tested antibiotics

Antibiotics/Unit	Class	No % Resistance	No %Susceptibility	No % Intermediate
Erythromycin 15 µg	Macrolide	36 (72%)	20%	8%
Clindamycin 2 µg	Lincosamide	39 (78%)	16%	6%
Cefoxitin 30 µg	Cephamycin	17 (34%)	66%	00
Amoxicillin	SS Penicillin	46 (92%)	8%	00
Imipenem 10µg	Carbapenem	5 (10%)	90%	00
Aztreonam 30 µg	Monobactam	35 (70%)	20%	10%
Ceftriazone 30 µg	Cephalosporin	7 (14%)	52%	34%
Ceftazidine 30 µg	Cephalosporin	19 (38%)	50%	12%
Cefotaxime 30 µg	Cephalosporin	11 (22%)	62%	16%
Amoxicillin/Clavulanic 20/10 µg	Clavunate	25 (50%)	50%	00

Note: SS-Semi synthetic

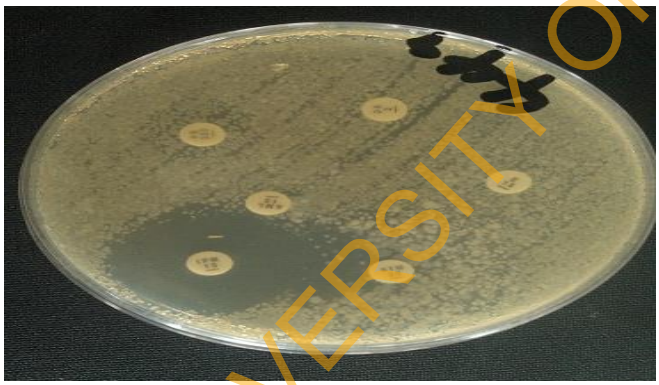

Fig. 1:
An observed synergy between Imipenem and Aztreonam in a *S. aureus* Strain

DISCUSSION

The rate at which resistance occurs among microbial populations is often driven by the overuse and abuse of antimicrobial agents in many clinical settings. Beta-lactam (β-Lactam) antibiotics are among the most frequently prescribed antibiotics worldwide, and as such their use is subject of the problems associated with microbial resistance (Shoaib *et al*., 2001). The development of resistance to β-lactam antimicrobials, often concurrently with resistance to other antimicrobial agents, poses a great challenge to the prevention and treatment of *S. aureus* infections (Arias, 2009; Llarrull *et al*., 2009).

From this study, 42% of the *Staphylococcus aureus* strains that were resistant to amoxicillin were susceptible to amoxicillin/clavulanic acid. This showed strong indication that 42% of the strains produces beta-lactamase that were inhibited by beta-lactamase inhibitor. For most bacterial species the incidence of β-lactamase production throughout the world are remarkably similar. Incidence of β-lactamase production in *Staphylococcus aureus* has consistently been reported to be over 80% in all parts of the world (Shoaib *et al.*, 2001). β-lactamase production is by far the most important and most widespread mechanism of resistance. It has extended to cover more and more β-lactam agents including third generation cephalosporins. β-lactam antibiotics such as penicillins, cephalosporins and cephamycins which all have similar core structure consisting of a β-lactam agents including third generation.

Table 2:
Antibiotic susceptibility/resistance pattern of tested MRSA strains. Diameter (mm) of zone of inhibition

S/N	E R≤13, S≥23, I 14-22	DA R≤14, S≥21, I 15-20	AML R≤28, S≥29	IPM R≤13, S≥16, I 14-15	ATM R≤17, S≥21, I 18-20	CRO R≤13, S≥21, I 14-20	AMC R≤19, S≥20	CAZ R≤14, S≥18, I 15-17	FOX R≤24, S≥25	CTX R≤14, S≥20, I 15-19
S. aureus	10	00	00	30	00	16	00	00	21	25
DF001	R	R	R	S	R	I	R	R	R	S
S. aureus	00	00	00	20	00	17	00	00	18	15
DF003	R	R	R	S	R	I	R	R	R	I
S. aureus	00	00	00	00	00	00	00	20	00	18
DF004	R	R	R	R	R	R	R	S	R	I
S. aureus	00	00	00	30	17	21	00	26	00	16
DF010	R	R	R	S	R	S	R	S	R	I
S. aureus	20	05	17	32	9	20	15	00	9	22
DF012	I	R	R	S	R	I	R	R	R	S
S. aureus	00	00	00	27	24	26	00	28	23	20
DF025	R	R	R	S	S	S	R	S	R	S
S. aureus	00	00	00	35	00	00	00	00	15	10
DF029	R	R	R	S	R	R	R	R	R	R
S. aureus	00	00	00	31	33	30	00	28	20	30
DF032	R	R	R	S	S	S	R	S	R	S
S. aureus	00	00	00	26	00	22	00	17	00	00
DF034	R	R	R	S	R	S	R	I	R	R
S. aureus	00	00	00	25	30	20	11	30	00	00
DF035	R	R	R	S	S	I	R	S	R	R
S. aureus	00	00	00	26	20	28	00	20	20	19
DF036	R	R	R	S	I	S	R	S	R	I
S. aureus	00	26	25	00	20	15	00	00	20	18
DF038	R	S	R	R	I	I	R	R	R	I
S. aureus	00	00	00	30	00	00	00	00	15	09
DF039	R	R	R	S	R	R	R	R	R	*R*
S. aureus	00	00	00	30	18	20	00	25	00	14
DF041	R	R	R	S	I	I	R	S	R	R
S. aureus	00	00	17	20	00	21	00	18	21	25
DF043	R	R	R	S	R	S	R	R	R	S
S. aureus	00	00	00	31	00	20	00	00	24	25
DF045	R	R	R	S	R	I	R	R	R	S
S. aureus	00	00	00	31	00	19	15	00	21	18
DF046	R	R	R	S	R	I	R	R	R	I

The relatively high percentage susceptibility to the beta-lactam antibiotics shown by this study reviewed the effectiveness of these antibiotics on the strain of the *Staphylococcus aureus* as seen in cefoxitin (66%), ceftriazone (52%), ceftazidime (50%), cefotaxime (62%) with the highest susceptibility in imipenem (90%). Imipenem is the carbapenem that is used in the treatment of infection caused by beta-lactamase producing microorganisms. However, imipenem is expensive and its use is closely guided to control bacterial resistance. Cefotaxime was highly resistant to staphylococcal β-lactamases and has a good activity against Gram positive and Gram negative bacteria (Neu *et al*., 1979). The observed 62% susceptibility observed in this study agrees with Garcia-Rodriguez *et al*., 1992 who observed high activity of cefotaxime against *Staphylococcus aureus*. Cefotaxime showed more activity than ceftriazone. This also agrees with a study by Willke and Tural 1987 who showed that cefotaxime was the most effective third generation cephalosporins as compared to that of ceftizoxime and ceftriaxone. (Shoaib *et al.*, 2001). Eltahawy *et al*., in 1988 showed that ceftriaxone and ceftazidime were more active than augmentin by inhibiting 90% of the *Staphylococcus aureus*. Frenkel *et al*., in 1988 also observed high antibacterial activity of ceftriaxone against *Staphylococcus aureus* by eradicating 97% of the test strains. The present result shows amoxicillin/clavulanic acid and other cephalosporins inhibited the tested strains at the same level. An interesting synergy was observed in two *S. aureus* strains in this study whereby the strains were susceptible to imipenem but resistant to aztreonam with susceptibility on the side of aztreonam facing imipenem. This is an indication that a combine therapy of imipenem and aztreonam will lead to enhanced antimicrobial activity.

There is a relatively high prevalence of MRSA in this study (34%). This observation supports the report by Kandle *et al.*, (2003) which showed 35% MRSA but slightly lower to reports by Tiwari *et al.*, (2008) who

observed 42.2% MRSA. All the MRSA in this study were resistant to clindamycin and erythromycin. This agrees with the clindamycin resistance of 92 % reported by Onanuga *et al.*, (2005). The MRSA were generally susceptible to imipenem and relatively susceptible to cephalosporins. This is contrary to the study by Weems, (2001) which reported that MRSA strains are equally resistant to all β-lactam antibiotics.

The observed relatively high prevalence of MRSA in the studied population calls for more survelliance study and implementation of effective policies for control of MRSA in hospitals and communities in Nigeria. The observed relative susceptibility of the studied *S. aureus* strains to third generation cephalosporin and imipenem is a clear indication of the effectiveness of some beta-lactam antibiotics against *S. aureus* strains. Thus, the existence of MRSA susceptible to these beta-lactam antibiotics may provide an opportunity for the recommendation of these drugs for empirical treatment and reducing pressure on other non-beta-lactam antibiotics

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