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Invitro Susceptibility test of *Staphylococcus* species Isolated from Sudanese Anterior Nares to different types of Antibiotics

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A B S T R A C T

The aim of this study was to evaluate the susceptibility of *Staphylococcus* species isolated from the anterior nares of different Sudanese population to various antibiotics. The bacteria were isolated and identified using cultural and biochemichal procedures. Twenty five *Staphylococcus* isolates were applied in this study including eleven species and two sub species.They were: *Staph.epidermidis, Staph.aureus, Staph.capitis, Staph.hyicus* (coagulase-positive), *Staph.hyicus* (coagulase-negative), *Staph.caseolyticus, Staph.simians, Staph.lugdunensis, Staph.delphini, Staph.schleiferi, Staph.hominis, Staph.capitis* sub spp ureolyticus and *Staph.Cohni* sub spp ureolyticus. One isolate for *Staph.hyicus* coagulase-positive, *Staph.cohni* sub spp ureolyticus and *Staph.schleiferi*, three isolates for *Staph.aureus, Staph.epidermidis* and two isolates for the rest.The identified isolates were subjected to sensitivity test using disc diffusion method. Seventeen types of antibiotics were used: vancomycin, cefuroxime, cefotaxime, methicillin, penicillin, ampicillin, cloxacillin, ciprofloxacin, gentamicin, erythromycin, tetracycline, streptomycin, colistin, nalidixic acid, chloramphenicol, co-trimoxazol and nitrofurantoin. All of the isolates were sensitive to vancomycin, cefuroxime, cefotaxime, ciprofloxacin, gentamicin, tetracycline, streptomycin, colistin, chloramphenicol and nitrofurantoin. Antibiotics resistance -patternes were reported as: 52% for methicillin, 44% for penicillin, 36% for co-trimoxazol, 20% for ampicillin, 20% for nalidicxic acid, 12% for cloxacillin and 4% for erythromycin. *Staphylococcus aureus* isolates were resistant to methicillin, penicillin and nalidixic acid. *Staph.epidermidis* isolates were resistant to penicillin and co-trimoxazol. *Staph.hyicus* coagulase-positive showed multi drug resistantce. Methicillin resistance was domenant in this study.

Keywords

Staphylococcus species, Antibiotics, Sensitivity test

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Introduction

Antimicrobial resistance can increase the morbidity, mortality and treatment cost of Staphylococcal infections (Hartman *etal.*, 1984). Multi drug resistance in pathogenic and opportunistic bacteria was increasingly documented. These bacteria pose life threatening risks to the hospitalized patients and their caregivers (Jones *et al.*, 2004). *Staphylococci* are one of the most numerous resistances to many prescribed antibiotics (Mun *et al.*, 2013). Both strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* have accumulated multiple resistance determinants (Archer *et al.*, 1994).

One mode of penicillin-resistant action of bacteria is by producing β-lactamase to destroy penicillins (Tenover, 2006). *Staphylococcus epidermidis* and other coagulase-negative *Staphylococci* (CoNS) are leading causes of surgical site and central-line-associated blood stream infections (Sievert *eT al.*, 2013and Otto, 2009). Little is understood about the mechanisms of pathogenesis and optimal treatment of *Staphylococcus epidermidis*.

Many of the clinical decisions made when treating this species are based on assumptions from studies in *Staphylococcus aureus. Staphylococcus aureus* is considered to be the most virulent and is the leading cause of healthcare - associated infections (*Klein et al.*, 2007). Coagulase-negative *Staphylococci* (CoNS) are frequently associated with catheter and prosthetic device infections. Antimicrobial therapy is essential for most Staphylococcal infections, and *in vitro* susceptibility testing plays a pivotal role in the selection of antimicrobial agents (Aldridge, 1995). For most Staphylococcal isolates, susceptibility to penicillinase - resistant penicillins (eg, oxacillin) is the most important result. Methicillin resistant *Staphylococcus aureus* (MRSA) becomes a prime nosocomial pathogen for patients in hospitals and nursing homes during the past ten years (Boyce, 2007, Chamber *et al*., 2009, Hardy *et al*., 2006 and Kallen *et al.*, 2010).

The use of antibiotics in humans and in animals (therapeutic, growth promotion and prophylactic) possibly led to the selective increase of resistance in bacterial populations (Suleiman *et al*., 2013). The methicillin resistance of *Staphylococci* is mediated by the mec A gene, which is carried by a mobile genetic element known as the Staphylococcal cassette chromosome mec (SCCmec) (Vanegas-López *et al.*, 2012). The penicillin binding protein 2a (PBP2a) has a reduced affinity for beta-lactam antibiotics, resulting in resistance to most beta-lactam antimicrobial agents (Mirzaei *et al*., 2012 and Yamada *et al*., 2013). The continued emergence of antimicrobial drug resistance is a serious problem for the antibiotic treatment of patients with Staphylococcal infections in the clinic. Studies by Kuehnert *et al*., (2006) reported that 60-85% of *Staphylococcus* strains isolated from clinical samples were resistant to methicillin. The major problem lies in the fact that infections caused by methicillin-resistant *Staphylococcus* strains (MRS) were difficult to treat. In some cases, the isolates were only susceptible to glycopeptides and new drugs, such as linezolid, tigecycline, daptomycin and quinupristin/dalfopristin (Critchley *et al*., 2003 and Otto, 2009). *Staphylococcus* species that was prevalent in animals associated with frequent resistance to methicillin was *Staphylococcus schleiferi* (Griffeth *et al*., 2008 and Kawakami *et al*., 2010). Coagulase-negative MRS species such as *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Staphylococcus sciuri*, and *Staphylococcus simulans* were isolated from animals.(Van Duijkeren *et al*., 2004, Feßler *et al.*, 2010 and Chah *etal*., 2014).

Materials and Methods

The bacterial isolation and identification were performed using traditional cultural procedures according to Barrow and Feltham, (2003).Media were obtained and prepared according to the methods described by Oxoid (Oxoid, Laboratories, London).Reagents were obtained from the British Drug House Chemicals (BDH ltd Poole, England). All biochemical confirmatory tests were performed according to Sneath *et al.*, (1986), Barrow and Feltham, (2003) and El Sanousi *et al.*, (2015).

Randomly 25 isolates were selected of 164 *Staphylococcus* positive results that were

isolated from a total of 200 nasal swab specimens taken from sudanese community including: Hospital staff, subject in contact with animals, subject worked in clean environments (not in contact with patients or animal) and children group with virtually 50 samples for each group. The isolates included eleven species and two sub species. These were: *Staph.epidermidis, Staph.aureus, Staph.capitis, Staph.hyicus* (coagulase-positive), *Staph.hyicus* (coagulase-negative), *Staph.caseolyticus, Staph.Simians, Staph. lugdunensis, Staph.delphini, Staph.schleiferi, Staph.hominis, Staph.capitis* sub spp ureolyticus and *Staph.cohni* sub spp ureolyticus. One isolate for *Staph.hyicus* coagulase-positive, *Staph.cohni* sub spp ureolyticus and *Staph.schleiferi*, three isolates for *Staph.aureus* and *Staph.epidermidis* and two isolates for the rest.

The identified isolates were subjected to susceptibility test using disc diffusion method according to Cheesbrough, (2000).A plate of nutrient agar was dried in the incubator for 30 minutes then a diluted suspension of the organism was poured onto the surface of the medium. Using sterile forceps, the antibiotic discs were gently applied on the plate and incubated at 37°C for 24 hours. The zones of inhibition were measured in millimetres using a ruler and defined according to the chart within the ranges.

Results interpretation was done in accordance with the zone size interpretative chart of the manufacturer. Susceptibility of *Staphylococcus* species were done to seventeen types of antibiotics: Ampicillin (10µg), co-trimoxazol (25.µg), gentamicin (10µg), streptomycin (10 µg), tetracycline (10µg), nalidixic acid (30µg), nitrofurantoin (200µg), colistin(25 µg), cloxacillin (5µg), chloramphenicol (10µg), erythromycin (5µg), penicillin(1 I.U), all of these were obtained from (Plasmatec Lab. Products Ltd, U.K). Ciprofloxacin (5 µg) (CIP/A LTD. Mumbae Central, India), cefuroxime (30µ g), Cefotaxime (30 µg), methicillin (10µ g) (MAST Diagnostics, Mast Group Ltd. Merseyside, U.K.) and vancomycin (30 µg) (Oxoid).

Results and Discussion

All of the isolate were sensitive to vancomycin, cefuroxime, cephotaxime, ciprofloxacin, gentamicin, tetracycline, streptomycin, colistin, chloramphenicol and nitrofurantoin (Table-1). *Staphylococcus epidremidis* showed large zones of inhibition to cephalosporins, ciprofloxacin and nitrofurantoin (Table-3).Antibiotics resistance patternes were reported as: 52% for methicillin, 44% for penicillin, 36% for co-trimoxazol, 20% for ampicillin, 20% for nalidixic acid, 12% for cloxacillin and 4% for erythromycin (Table-2). *Staphylococcus aureus* were resistant to methicillin, penicillin and nalidixic acid. *Staphylococcus epidermidis* isolates resisted penicillin and co-trimoxazol, *Staphylococcus hominis* resisted methicillin and penicillin while *Staphylococcus caseolyticus* resisted methicillin and nalidixic acid. *Staphylococcus hyicus coagulase*-positive gave multi drug resistantce results. Methicillin resistance was reported in nine species among these isolates (Table -2).

The identified isolates were subjected to the susceptibility test using disc diffusion method to seventeen types of antibiotics. Cefuroxime, cefotaxime, ciprofloxacin, gentamicin, tetracycline, streptomycin, vancomycin, nitrofurantoin, colistin and chloramphenicol gave different degrees of inhibition to all isolates.

Most of the isolates were resistant to penicillin and methicillin. *Staphylococcus aureus* isolates showed resistant pattens to

methicillin, penicillin, and nalidixic acid. *Staphylococcus epidermidis* isolates were resistant to penicillin and co-trimoxazol.

Methicillin resistance was reported in nine species which were comparable with a result obtained by Phophi, *et al.*, (2019) on antimicrobial resistance patterns of coagulase – negative *Staphylococcus* species included: *Staphylococcus epidermidis, Staphylococcus hominis* and *Staphylococcus hyicus*. This study revealed that the majority of coagulase negative *Staphylococci* (CoNS) gave high rate of sensitivity to cefoxitin and vancomycin. Most of them were β-lactam resistant in particular to penicillins and ampicillin. Our study agreed with a previous study performed by Lee *et al.*, (2006) who evaluated the suitable antibiotics used for the treatment of catheter- related infections caused by *Staphylococci.* The study conducted proved that 5mg/ml of vancomycin and ciprofloxacin can eradicate *Staphylococcus aures* and *Staphylococcus epidermidis* within five days, while complete eradication was not achieved with erythromycin and other drugs under investigation.

Table.1 Antibiotics susceptibility patterns of *Staphylococcus* species.

Antibiotics	Sensitive zone: 1.2 - ∞ mm No. of isolates	Intermediate zone: 0.4 – 1.1mm No. of isolates	Resistance zone: ≤ 0.3 No. of isolates	Percentages of sensitivity %
Cefotaxime (30 µg)	18	7	-	100%
Cefuroxime (30 µg)	19	6	-	100%
Cloxacillin (5 µg)	15	7	3	88%
Ampicillin (10 µg)	11	9	5	80%
Penicillin (1i.u.)	7	7	11	56%
Methicillin (10 µg)	6	6	13	48%
Nitrofurantoin (200 µg)	21	4	-	100%
Ciprofloxacin (5 µg)	20	5	-	100%
Gentamicin (10 µg)	19	6	-	100%
Streptomycin (10 µg)	13	12	-	100%
Tetracycline (10 µg)	12	13	-	100%
Vancomycin (30 µg)	11	14	-	100%
Chloramphenicol(10 µg)	9	16	-	100%
Colistin (25 µg)	8	17	-	100%
Erythromycin (5 µg)	14	10	1	96%
Nalidixic acid(30 µg)	8	12	5	80%
Co-trimoxazol(25 µg)	8	8	9	64%

Table.2 Antibiotics resistant species (zone of inhibition: ≤ 0.3mm)

***Staphylococcus* resistant species**	**Antibiotics**	**No. of the isolates**	**Percentages of Resistant (%)**
***Staph.hyicus* coagulase -positive**	Erythromycin (5 µg)	1	4
***Staph.hyicus* coagulase- positive** ***Staph.hyicus* coagulase- negative**	Cloxacillin (5 µg)	1 2	12%
Staph.hyicus* coagulase -negative** ***Staph.hyicus* coagulase- positive** ***Staph.delphini ***Staph.cohni* sub spp ureolyticus**	Ampicillin (10 µg)	1 1 2 1	20%
Staph.cohni* sub spp ureolyticus** ***Staph.hyicus* coagulase -positive** ***Staph.caseolyticus ***Staph.aureus***	Nalidixic acid (30 µg)	1 1 2 1	20%
Staph.lugdunensis ***Staph.delphini*** ***Staph.epidermidis*** ***Staph.hyicus* coagulase -positive** ***Staph.simians***	Co-trimoxazol (25 µg)	2 2 2 1 2	36%
Staph.hominis ***Staph.aureus*** ***Staph.hyicus* coagulase -negative** ***Staph.hyicus* coagulase- positive** ***Staph.delphini*** ***Staph.epidermidis*** ***Staph.cohni* sub spp ureolyticus**	Penicillin (1i.u.)	1 2 2 1 2 2 1	44%
Staph.hyicus* coagulase -negative** ***Staph.hyicus* coagulase -positive** ***staph.delphini ***staph.capitis*** ***Staph.capitis* sub spp ureolyticus** ***Staph.cohni* sub spp ureolyticus** ***Staph.hominis*** ***Staph.caseolyticus*** ***Staph.aureus***	Methicillin (10 µg)	2 1 2 1 1 1 1 2 2	52%

Table.3 Antibiotics highly sensitive species (zone of inhibition: ≥ 1.6 mm)

Staphylococcus spps	Antibiotics	No of isolates	Percentages %
Staph.epidermidis ***Staph.cohni* sub spp ureolyticus** ***Staph.hominis*** ***Staph.caseolyticus*** ***Staph.simians***	Cefotaxime (30 µg)	2 1 1 2 2	32%
Staph.epidermidis ***Staph.capitis*** ***Staph.cohni* sub spp ureolyticus** ***Staph.caseolyticus*** ***Staph.simians***	Cefuroxime (30 µg)	2 1 1 1 2	28%
Staph.hyicus* coagulase - negative** ***Staph.epidermidis ***Staph.hominis*** ***Staph.caseolyticus*** ***Staph.simians***	Ciprofloxacin (5 µg)	1 1 1 1 1	20%
Staph.hominis ***Staph.lugdunensis*** ***Staph.delphini*** ***Staph.epidermidis***	Nitrofurantoin (200 µg)	1 1 1 1	16%
Staph.hominis ***Staph.simians*** ***Staph.delphini***	Gentamicin (10 µg)	1 1 1	12%
Staph.hyicus* coagulase – positive** ***Staph.simians	Streptomycin (10 µg)	1 1	8%
Staph.simians ***Staph.hyicus* coagulase- positive**	Chloramphenicol (10 µg)	1 1	8%
Staph.hominis ***Staph.simians***	Erythromycin (5 µg)	1 1	8%
Staph.simians	Cloxacillin (5 µg)	2	8%
Staph .simians	Ampicillin (10 µg)	2	8%
Staph.simians	Tetracycline (10 µg)	1	4%

Methicillin resistant *Staphylococcus aureus* was shown in (8%) of the isolates under the study. This result was comparable with a result carried out by Tigabu *et al.*, (2018) whom isolated and identified methicillin resistant *Staph aureus* (MRS) in 14 (9.7%) of 143 (23%) isolates of *Staphylococcus aureus* from a total of 622 nasal swab specimens collected from school children in Ethiopia. The study also reported that, gentamicin, clindamycin, and ciprofloxacin were the most effective antibiotics whereas penicillin and tetracycline were not effective.

The study concluded that different *Staphylococcus* species under the *in vitro* antibiotic susceptibility test showed variable degrees of sensitivity to cephalosporins, vancomycin, ciprofloxacin, gentamicin, tetracycline, streptomycin, chloramphenicol, nitrofurantoin and colistin, while they were resistant to methicillin, penicillins, ampicillin, cloxacillin, nalidixic acid, co-trimoxazol and erythromycin

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