ISOLATION OF ANTIBIOTIC-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI FROM HOSPITALIZED PATIENTS IN KHARTOUM TEACHING HOSPITAL

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KEY WORDS: Antibiotic-resistant, Coagulase-negative Staphylococci, Susceptibility test.

ABSTRACT

A total of 188 clinical specimens were collected from hospitalized patients in Khartoum Teaching Hospital. 68 coagulase-negative staphylococci were isolated. These were identified as *S. epidermidis* 33 (48.5%), *S. saprophyticus* 12(17.7%), *S. haemolyticus* 12(17.7%), *S. lugdunensis* 6(8.8%), and *S. schleiferi* 5(7.3%). All isolates were studied for their susceptibility to traditionally used antibiotics. The results revealed that 93.6% of the isolates were resistant to beta-lactam antibiotics, and 72.3% were resistant to a wide range of selected antibiotics when judged by NCCLS criteria using disk diffusion method on Mueller-Hinton agar. It is concluded that resistance to antibiotics is common in Sudan.

الملخص

جمعت 188 عينة إكلينيكية من مرضى بمستشفى الخرطوم التعليمي، عزل من هذه العينات 68 من المكورات العنقودية السالبة لإنزيم التلزن وتم التعرف على هذه السلالات على أنها المكورات العنقودية البشروية 33(48.5%)، المكورات العنقودية المتعايشة 12 (17.7%)، المكورات العنقودية الحالة للدم 12(17.7%)، المكورات العنقودية اللقدننسية 6(8.8%)، المكورات العنقودية الإسكلفرية 5(7.3%). جميع هذه العزلات درست حساسيتها للمضادات الحيوية المستخدمة في الحالات المرضية. أظهرت النتائج أن 93.6% من العزلات مقاومة لعائلة البيتالاكتام وأن 72.3% منها مقاومة لعدد من المضادات الحيوية المختارة لهذه الدراسة وذلك عندما أجريت تجربة الحساسية بطريقة الإنتشار من الأقراص على وسط مولر-هنتون. يستخلص من هذه الدراسة أن المقاومات للمضادات الحيوية شائعة في السودان.

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INTRODUCTION

The family Micrococcaceae consists of four genera: *Planococcus*, *Stomatococcus*, *Micrococcus* and *Staphylococcus*. *Staphylococcus* is a significant human pathogen, causing a wide spectrum of diseases ranging from superficial cutaneous to life-threatening systemic maladies (Murray *et al.*, 1994).

Members of the genus *Staphylococcus* are Gram-positive cocci (0.5 to 1.5 μm in diameter) that occur singly, in pairs, tetrads, short chains (three or four cells), and irregular "grape-like" clusters. Ogston introduced the name "Staphylococcus" (from staphyle, a bunch of grapes) for the group of micrococci causing inflammation and suppuration. Rosenbach used the term in a taxonomic sense and provided the first description of the genus *Staphylococcus* (Balows *et al.*, 1992).

A total of 27 species and 7 subspecies are currently recognized in the genus *Staphylococcus*, with 14 species and 2 subspecies found in humans. The species most commonly associated with human infection are *Staphylococcus aureus* (*S. aureus*) (the most virulent and producer of coagulase), *S. epidermidis*, *S. haemolyticus*, *S. lugdunensis*, *S. saprophyticus*, and *S. scheleiferi* (are commonly referred to as "coagulase-negative staphylococci (CoNS)") (Murray *et al.*, 1994).

In the past CNS were generally considered to be contaminants having little clinicl significance. Over the past 2 decades, however, these organisms have become recognized as important agents of human disease (Koneman *et al.*, 1992).

MATERIALS AND METHODS

In this study, 188 clinical specimens were collected from hospitalized patients in Khartoum Teaching Hospital (KTH). These included: 33 from superficial incisionals, 40 from deep (open) infected wounds, 13 from decubitus ulcers, 41 of pus, 30 of midstream urine, and 31 of catheterized urine.

All specimens were inoculated onto blood agar (Oxoid) as enriched medium, Mac Conkey agar (Oxoid) as differential medium and mannitol salt agar (Difco) as selective medium. The plates were incubated at 35-37 °C for 16-24 hours for primary isolation. The

isolates of interest were judged by coagulase test. The isolates were identified according to Barrows and Feltham (1993).

Antibiotic susceptibility pattern was carried out using disc diffusion method, performed according to the National Committee for Clinical Laboratory Standards (NCCLS), on Mueller-Hinton agar (Oxoid). Antibiotics used were Penicillin G, Amoxicillin, Augmentin, Cephalexine, Cephraddine, Methicillin, Co-trimoxazole, Gentamicin, Erythromycin, Clindamycin, Ciprofloxacin, and Vancomycin. A suspension of the test organisms was matched to 0.5 McFarland standard turbidity tube and inoculated onto media, then incubated at 35-37 °C for 16-18 hours, and examined for evidence of the growth.

RESULTS

The majority of the specimens from which the coagulase-negative staphylococci were isolated include: superficial incisionals 16, deep (open) infected wounds 18, decubitus ulcers 4, pus 8, midstream urine 8, and catheterized urine 14.

The frequencies and percentage of the isolated CoNS is shown in (Table 1). The specimens from which the *S. epidermidis* were most frequently isolated were as follows: superficial incisionals 3, deep (open) infected wounds 8, decubitus ulcers 4, pus 8, and catheterized urine 10. Most of the *S. saprophyticus* were isolated from midstream urine 8, and catheterized urine 4. The majority of the *S. haemolyticus* were isolated from superficial incisionals 7, and deep (open) infected wounds 5. The specimens from which the *S. lugdunensis* were isolated were as follows: superficial incisionals 3, and deep (open) infected wounds 3. The majority of the *S. schleiferi* strains were isolated from superficial incisionals 3, and deep (open) infected wounds 2.

Table 1: The Species of Coagulase-negative Staphylococci Isolated in This Study

Organism	No.(%)
Staph. epidermidis	33(48.5)
Staph. saprophyticus	12(17.7)
Staph. haemolyticus	12(17.7)
Staph. lugdunensis	6(8.8)
Staph. schleiferi	5(7.3)

The overall resistance rate of CoNS to beta-lactam antibiotics and other selected antibiotics was 93.6% and 72.3% respectively. All isolates (100%) were resistant to penicillin G and methicillin, 95.6% to amoxicillin and augmentin, 85.3% to cephalexine and cephradine, 64.7% to co-trimoxazole, 66.2% to gentamycin and erythromycin, 61.8% to ciprofloxacin, 94.1% to clindamycin, and 80.9% to vancomycin. The resistance pattern of CoNS to beta-lactam antibiotics is shown in (Table 2), and to the other antibiotics is shown in (Table 3).

Table 2: The Resistance Pattern of the Isolated Coagulase-negative Staphylococci to Beta-lactam Antibiotics

Beta-lactam Antibiotics	No.(%)				
	S. epidermidis	*S. saprophyticus*	*S. haemolyticus*	*S. lugdunensis*	*S. scheleiferi*
Penicillin G	33(100)	12(100)	12(100)	6(100)	5(100)
Amoxicillin	31(94.0)	12(100)	11(91.7)	6(100)	5(100)
Agumentin	33(100)	11(91.7)	12(100)	5(83.3)	4(80.0)
Cephalexine	29(87.9)	9(75.0)	10(83.3)	6(100)	4(80.0)
Cephradine	30(90.0)	9(75.0)	9(75.0)	6(100)	4(80.0)
Methicillin	33(100)	12(100)	12(100)	6(100)	5(100)

Table 3: The Resistance Pattern of the Isolated coagulase-negative Staphylococci to other Antibiotics

Other Antibiotics	No. (%)				
	S. epidermidis	*S. saprophyticus*	*S. haemolyticus*	*S. lugdunensis*	*S. schleiferi*
Cotrimoaxazole	24(72.7)	8(66.7)	6(50.0)	4(66.7)	2(40.0)
Gentamicin	25(75.7)	8(66.7)	5(41.7)	4(66.7)	3(60.0)
Erythromycin	26(78.8)	9(75.0)	6(50.0)	3(50.0)	1(20.0)
Ciprofloxacin	21(63.6)	12(100)	4(33.3)	3(50.0)	2(40.0)
Clindamycin	29(87.9)	12(100)	12(100)	6(100)	5(100)
Vancomycin	27(81.8)	11(91.7)	10(83.3)	4(66.7)	3(60.0)

On the other hand, the isolated CoNS showed intermediate sensitivity to antibiotics: cephalexine 8, cephradine 7, co-trimoxazole and erythromycin 12, ciprofolxacin 11, gentamicin and vancomycin 8, amoxicillin 2, clindamycin and augmentin 1.

There was a rate of 2.2% sensitivity of isolated CoNS to beta-lactam antibiotics. The rate of sensitivity to the remaining antibiotics was 14.9%. The number sensitive to co-trimoxazole 12(17.6%), gentamycin and ciprofloxacin 15(22.0%), erythromycin 11(16.2%), clindamycin 3(4.4%) and vancomycin 5(7.4%).

DISCUSSION

Hospital-acquired infections happened during hospitalization of the patients. The main finding in this study was the relatively increased incidence of nosocomial infections caused by coagulase-negative staphylococci in Khartoum General Hospitals. There were considered, for many years, as commensal in the skin, but are now recognized as major agents of nonsocomial infections. Bacterial factors (increased resistance), host factors (immune status) and multiplication at the portal of entry (presence of foreign material), have contributed to the increased incidence of nosocomail infections (Herard *et al.*, 1998).

In this study, the overall rate of antibiotic resistance of CoNS was 83.0%, and this poses a great opportunity to the spread of these organisms in the hospital environment. Because most infections due to CoNS are nosocomial, it is not surprising that they have increasingly aquired resistance to multiple antibiotics, such as in *S. aureus*. About 80% to 90% of the isolated CoNS from human specimens produce an inducible beta-lactamase(Christof *et al.*, 2001). Furthermore 60% to 80% of nosocomial CoNS are methicillin-resistant. Methicillin resistance clinically precludes therapy with available beta-lactam antibiotics(Christof *et al.*, 2001). In addition, methicillin resistant strains are often resistant to macrolides, lincosamides, aminoglycosides, fluoroquinolones, and other antimicrobial agents. Therefore the emergence of the CoNS strains with reduced susceptibility to glycopeptides has raised concern about the development of resitant organism to all antibiotics (Christof *et al.*, 2001).

Due to very high rate resistance of beta-lactam antibiotics in 68 CoNS isolates, the majority were resistant to penicillin and methicillin, these results showed that neither penicillin nor methicillin can be used in the treatment of infections caused by CoNS. A study carried out in England, showed the emergence of penicillinase producing staphylococci that rendered benzylpenicillin ineffective, and stimulate the search for penicillinase-resistant isolates. Penicillin, methicillins, and nafcillin followed by the acid-stable isoxazolyl penicillins, are now currently a major antibiotcs

used to treat hospital sepsis due to CoNs (Nathwani and Wood, 1993).

Recent studies indicated the emergence resistance of CoNS to the quinolones, particularly to ciprefloxacin (Raad *et.al.*, 1998). Tolerance and occasional resistance to vancomycin have been reported recently (Raad *et.al.*, 1998). In addition, several reports indicated that vancomycin and other glycopeptide antibiotics lose their effectiveness against *S. epidermidis* embedded in the biofilm environment on the surface of devices. Alternative agents have been proposed in the prevention and medical treatment of device-related and glycopeptides-tolerant *S. epidermidis* infections, these include: minocyclin, rifampin, and more recently quinupristinl/dalfopristin and the oxazolidinones (Raad *et.al.*, 1998).

The distribution of CoNS in skin lesion was investigated in England. *S. epidermidis, S. lugdurensis, S. haemolyticus, S. capitic*, and *S. hominis* were identified and tested for susceptibility to nine antimicrobials (benzylpenicillin, ampicillin, piperacillin, cefazolin, erythromycin, minocyclin, gentamycin, vancomycin, and ofloxacin). *S. lugdunensis* was the most susceptible to the nine tested antimicrobials. *S. epidermidis* was the least susceptible, *S. haemolyticus* also showed low susceptibility to all nine antimicrobials. Low susceptibility to penicillin may be explained by beta-lactamase production. The existence of CoNS especially concerning their potential pathogenicity and multiple drug resistance should not be neglected (Higaki *et al.*, 1999).

In this study, as may be seen in the results, the most effective antibiotics against CoNS were gentamicin and ciprofloxacin with quite low sensitivity. Thus the treatment of CoNS infections is difficult. Previous studies revealed that antimicrobials decreased susceptibility was found to be amongest the CoNS isolated, mainly 2 isolates *S. epidermidis* were decreased susceptibility to vancomycin by a disk diffusion method. However, the isolates were intermediate by MICV (8-6 μg/ml), this indicates the emergence of vancomycin resistant among CoNS, because they are usually multi-drug resistant (Garret *et al.*, 1999). Another study from India showed maximum sensitivity of CoNS to ciprofloxacin followed by norfloxacin and chloramphenicol (Deepack *et al.*, 1999). Whereas a report from

United Kingdom revealed a decline in susceptibility of CoNS to ciprofloxacin from 99.4% of 658 cultures examined in 1987 to 92.6% of 433 cultures studied in 1989 (George *et al.*, 1990).

In conclusion, nosocomial infections caused by CoNS is relative of incidence in Sudan. Antimicrobial resistance was found to be common among the CoNS isolates. Treatment of infections with CoNS is complicated because resistance and multi-resistance to antibiotics were increasingly prevalent.

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