Profile of Antibiotic Sensitivity and Resistance of some Pathogenic Bacteria Isolated from Clinical Specimens in Sudan

Asjad M. Mukhtar[1] and Humodi A. Saeed[1*]

[1]College of Medical Laboratory Science, Sudan University of Science and Technology, P O Box 407; Tel. +249 83 773074; Fax +249 83 771512; Khartoum, SUDANCorrespondence to Dr. Humodi Ahmed Saeed, E-mail: saeed@sustech.edu

Abstract:

In this study the activity of gentamicin, ciprofloxacin, ceftazinime impenem, amikacin, tetracycline, penicillin, nalidixic acid and nitrofurantoin has been evaluated. A total of 380 clinical specimens were collected from different hospitals in Khartoum state. Different types of bacteria were isolated. These include *Staphylococcus aureus* (n=37), *Escherichia coli* (n=70), *Klebsiella pneumoniae* (n=15), *Proteus spp.* (n=57) and *Pseudomonas aeruginosa* (n=67).

The study revealed that the profile of antibiotics sensitivity and resistance were as follows: Gentamicin 90.8 and 9.2%, ciprofloxacin 72.1 and 27.9%, ceftazinime 100.0 and 0.0%, impenem 100.0 and 0.0%, amikacin 94.0 and 6.0%, tetracycline 47.7 and 52.3%, nalidixic acid 65.9 and 34.1% and nitrofurantoin 86.3 and 13.7%, penicillin 16.7 and 83.3% and erythromycin 75.9 and 24.1% respectively.

The study concluded that ceftazidime and impenem were the most effective antibiotics against *Pseudomonas aeruginosa* isolated from wounds.

Key words: Antibiotic sensitivity and resistance**,** pathogenic bacteria.

الملخص:

في هذه الدراسة تم تقييم فاعلية الجنتاميسين والسبروفلوكساسين والسيفتازيديم والإمبينيم والإميكيسين والتتراسايكلين والبنسلين والناليديكسيك أسيد والنايتروفيورانتوين مع مجموعة مختلفة من البكتيريا الممرضة. جمعت 380عينة اكلينيكية من من مرضى يعانون من عدوى الجهاز البولي والتهاب الجروح والتهاب الأذن الوسطى بمستشفيات ولاية الخرطوم، تم عزل وتحديد أنواع مختلفة من البكتريا شملت العنقودية الذهبية (37) عتره والأسكريشية القولونية (70) عتره و الكلبسيلا (18) عتره والمتقبلة (57) عتره والزائفة الزنجارية (67) عتره. أظهرت هذه الدراسة أن مُرْتَسَم الحساسية والمقاومة للمضادات الحيوية على النحو التالي: الجنتاميسين 90,8 و 9,2% والسبروفلوكساسين 72,1 و 27,9% والسيفتازيديم 100,0 و 0,0 % والإمبينيم 100 و 0,0 % والإميكيسين 94,0 و 6,0 % والتتراسايكلين 47,7 و 52,3% والناليديكسيك أسيد 65,9 و34,1% والنايتروفيورانتوين 86,3 و 13,7 والبنسلين 16,7 و 83,3 % و الإريثرومايسين 75,9 و 24,1% على التوالي.

خلصت الدراسة الي أن السيفتازيديم والإمبينيم هما أفضل المضادات الحيوية الفاعلة ضد الزائفة الزنجارية .

Introduction

Antibiotic resistance is known world-wide. The widespread use of antibiotics for treatment of bacterial infections has lead to the emergence of resistant human pathogens (Van Winkelhoff, *et al.*, 1999).

Antibiotic resistance has moved on. Increasing numbers of antibiotic resistance occurs in gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and others. The problem of antibiotic resistance also occurs in gram-negative bacteria such as *Ps. aeruginosa*, *Escherichia coli* and others including many infections acquired in the hospitals and community.

Great differences have been documented between hospitals in the Sudan in the levels of resistant pathogens (Saeed and Eidha, 2003; Ahmed, 2005 and Shakak, 2006).

Despite the importance of antibiotics in human life, very little researches were carried out in hospitals in Khartoum State.

This study essentially designed to investigate antibiotics sensitivity and resistance profile of some important pathogenic bacteria.

Materials and Methods

This study was carried out in Khartoum Teaching Hospital, Ear, Nose & Throat Hospital, Ibrahim Malik Hospital, Medical Military Hospital and National Health Laboratory. Patients suffering from wound infections, ear infections and urinary tract infections were included in this study.

Antibiotic discs

The following antibiotic discs were obtained from Bioanalyse Ltd. UK: Amikacin (AK), 30 µg: Ceftazidime (CAZ), 30 µg: Ciprofloxacin (CIP), 5 µg: Erythromycin (E), 15µg: Gentamicin (CN) 10µg: Nalidixic acid (NA), 30µg: Nitrofurantoin (F), 300µg: Penicillin (P), 10 Units: Tetracycline (T), 30µg and Impenem (IMP), 10µg.

Collection and culture of specimens

The specimens were collected from patients hospitalized in five hospitals in Khartoum state (Khartoum Teaching Hospital, Medical Military Hospital, Ibrahim Malik Hospital, National Health Laboratory and ENT Hospital)

Patients' age ranged from less than a year to 92 years. Specimens from patients with ear and wound infections were collected by sterile cotton wool swabs. Urine samples were collected in sterile universal containers. These specimens were labeled with patient's name, age and code and then transported to the laboratory within 1/2 hour of collection. The specimens were inoculated on plates of blood agar; MacConkey agar and CLED agar (Oxoid Co. Ltd., U.K.), which were incubated aerobically at 37˚C for 18-24 hours. At the end of incubation period, plates were examined for

fermentation on MacConkey agar and CLED agar and haemolysis on blood agar. The morphological characteristics such as size, shape, color, pigment production and odors were studied.

Identification of bacterial isolates was based on cultural characteristics, Gram stain and biochemical tests (Cowan and Steel, 2003).

Sensitivity Test

The inoculum for sensitivity test was prepared by suspending three colonies of similar appearance using sterile loop in 2ml saline; the suspension was adjusted to McFarland turbidity standard by adding more bacteria or more saline (Vandopitte *et al.,* 199l). The antibiotics used were gentamicin, amikacin, ciprofloxacin, impenem and ceftazidime for *Ps. aeruginosa*. Nalidixic acid and nitrofurantoin for urinary isolates of enterobacteriaceae, (except *Proteus* spp.). Tetracycline, ciprofloxacin and gentamicin for all isolates except *Proteus spp*.

Seeding of the plates

Cotton wool swab previously immersed in suspension of the test organisms was rotated and squeezed to remove the excess and streaked evenly on dry Muller- Hinton agar (Oxoid Co. Ltd., U.K.). Seeded plates were left for few minutes at room temperature with lid closed (Vandopitte *et al.,* 199l).

Discs application

Applications of antibiotic discs were done by using pair of sterile forceps. The plates were left for few minutes for diffusion of antibiotics, then incubated for 24 hours at 35°C (Vandopitte *et al.,* 199l).

Interpretation of results

Inhibition zones of antibiotics were measured using a ruler. The diameter of zones of inhibition of test and control strains were interpreted using table (2) of NCCLs for test organism and table (3) of NCCLs for standard strains then recorded (Vandopitte, *et al.,* 1991 and WHO, 1997).

Results

Three hundred and eighty clinical specimens were collected from patients (males and females). These specimens included urine and ear and wound swabs (Table 1). Of the urine specimens, 82 (88.2%) gave bacterial growth when cultivated on CLED agar. Among these 56 (53.3%) were lactose fermenters (LF) and the rest 26 (46.7%) were non- lactose fermenters (NLF). Of ear swabs 66 (68.8%) gave bacterial growth and of wound swabs 99 (76.1%) gave bacterial growth.

Bacterial species isolated during this study

Aerobic culture of the swabs and urine yielded different bacterial species as follows:

Pseudomonas aeruginosa (67), *Escherichia coli*, (70), *Klebsiella pneumoniae* (15), *Proteus spp* (57) and *Staphylococcus aureus* (37) (Table 2).

Sensitivity test

All isolated bacteria were subjected to antimicrobial sensitivity test using modified Kirby- Bauer disc diffusion method.

The results revealed that the resistance rates of *E.coli* was 3.8% to gentamicin, 30% to ciprofloxacin, 9.1% to nitrofurantoin, 31.8% to nalidixic acid & 51.4% to tetracycline. The resistance rate of *K. pneumoniae* was 20% to gentamicin, 46.7% to ciprofloxacin, 20% to nitrofurantoin, 40% to nalidixic acid and 20% to tetracycline. The resistance rate of *Proteus spp* was 0% to gentamicin, 8.7% to ciprofloxacin & 16.6% to nalidixic acid. Resistance rate to *Ps. aeruginosa* was 11.9% to gentamicin, 4.5% to amikacin, 16.9% to ciprofloxacin and 0% to impenem and ceftazidime. Finally, resistance rate to *Staph. aureus* was 5.6% to gentamicin, 11.1%, 16.7% to erythromycin, and 83.3% to penicillin 47.2%.

Table 1. The frequency of bacterial isolates according to the specimens

Specimen	*Bacterium isolated*	*n(%)*
ne	*Ps. aeruginosa*	*8(10.0)*
	E. coli	44(54.3)
	K. pneumoniae	10(12.3)
	Proteus spp	18(22.2)
Wound swab	*S. aureus*	*1(1.2)*
	Ps. aeruginosa	*37(37.4)*
	E. coli	*14(14.1)*
	K. pneumoniae	*2(2.0)*
	Proteus spp.	*18(18.2)*
Ear swab	*S. aureus*	*22(33.3)*
	Ps. Aeruginosa	*28(28.3)*
	E. coli	*12(18.2)*
	K. pneumoniae	*3(4.6)*
	Proteus spp	*21(31.8)*
	S. aureus	8(12.1)

Key: *E.c. =Escherichia coli; K.pn.=Klebsiella pneumoniae; Pr.spp=Proteus spp; Ps.a=Pseudomonas aeruginosa;* S.a *=Staphylococcus aureus*

Table 2. Profile of antibiotic sensitivity and resistance against athogenic bacteria

Antibiotic	Bacteria	Source	S %	R %
Gentamicin	*Ps.a.*(n=67)	Wound	86.6	13.4
	E.c.(n=70)	wound, ear	94.2	05.8
	K.pn.(n=5)	wound, ear	80.0	20.0
	Pr. spp.(n=39)	urine, ear, wound	98.7	01.3
	S.a.(n=37)	ear, wound	94.4	05.6
Ciprofloxacin	*Ps.a.*(n=67)	Wound	82.1	17.9
	E.c.(n=70)	Ear, Wound	67.1	32.9
	K.pn.(n=5)	Ear, Wound	53.3	46.7
	Pr. spp.(n=39)	urine, ear, wound	91.9	08.1
	S.a.(n=37)	ear, wound	86.1	13.9
Ceftazideime	*Ps. a.*(n=67)	wound	100.0	00.0
Impenem	*Ps. a.*(n=67)	wound	100.0	00.0
Amikacin	*Ps. a.*(n=67)	wound	94.0	06.0
Tetracycline	*E.c.* (n=70)	Ear, wound	43.6	56.4
	K.pn.(n=15	Ear, wound	50.0	50.0
	S.a.(n=36)	ear, wound	50.0	50.0
Nalidixic acid	*E.c.* (n=44)	Urine	65.9	34.1
Nitrofurantoin	*E.c.* (n=44)	Urine	86.3	13.7
Penicillin	*S.a.*(n=36)	ear, wound	16.7	83.3
Erythromycin	*S.a.*(n=36)	ear, wound	75.9	24.1

Key: *E.c. =Escherichia coli; K.pn.=Klebsiella pneumoniae; Pr.spp=Proteus spp; Ps.a=Pseudomonas aeruginosa;* S.a *=Staphylococcus aureus;* S= sensitive; R= resistance

Discussion

This study undertaken to evaluate the antibiotics (gentamicin, amikacin, ceftazidime, impenem and ciprofloxacin) sensitivity and resistance profile of common pathogenic bacteria (n=246) isolated from clinical specimens. The selection of these antibiotics was done according to World Health Organization recommendations.

In this study all *Ps. aeruginosa* strains (n=67) were found sensitive (82.1-100%) when tested against gentamicin, amikacin, ceftazidime, impenem and ciprofloxacin. The susceptibility for each antibiotic was as follows; gentamicin, 86.6%; amikacin, 94.0; ceftazidime and impenem, 100% each and ciprofloxacin, 82.1%. This results in line with the results that obtained by Rhodora, (1988), Lutfu, *et al*., (2004), Shenoy, *et al.*, (2002) and Zakaria (2005) respectively.

As *Ps. aeruginosa* isolated from patients attended four leading hospitas and one national reference laboratory distributed over large area in Khartoum state, these results indicated that the antibiotics commonly used in treatment of urinary tract, wound and ear infections are still effective.

E. coli (n=70) were tested against gentamicin, ciprofloxacin and nalidixic acid. The later for urinary *E. coli* only. The results revealed the sensitivity and resistance profile were range from 65.9 to 94.2 and 34.1 to 5.8 respectively. The sensitivity and resistance profile of *E. coli* was almost similar to that reported by Kadri *et al.*, (2004) who found that *E. coli* was significantly resistance to the commonly used antibiotics. Furthermore, *Proteus* spp.(n=39) isolated from urine, ear and wound. The isolates were tested against gentamicin and ciprofloxacin. The profile of antibiotic sensitivity and resistance was found 98.7% to 01.3% and 91.9% to 08.1% respectively. The two antibiotics were tested against *Staphylococcus aureus* (n=37) but the source of these isolates was ear and wound. The results indicated of antibiotic sensitivity and resistance as follows; 94.4% to 05.65 and 86.1% to 13.9% respectively. The rate of resistance increased (20.0%to 46.7%) in *Klebsiella pneumoniae* (n=5) which isolated from the same source (wound, ear).

Resistance of *Pseudomonas aeruginosa* (n=67) to ceftazideime (0.0%), impenem (0.0%) and amikacin (6.0%) is rather low. This result almost similar to the study of Randrianirina *et al;* (2007) in Madagascar. Moreover, approximately, 50% sensitivity and resistance profile (43.6% and 56%; 50.0% and 50.0% and 50.0% and 50.0%) was reported to tetracycline when tested against *Escrichia coli*, (n=70), *Klebsiella pneumoniae* (n=5) and *Staphylococcus aureus* (n=37) respectively. On the other hand, no resistance reported in this study when evaluated ceftazideime and impenem against *Pseudomonas aeruginosa* but only 06.0% of resistance of amikacin against the same organism. It is concluded that ceftazidime and impenem were the most effective antibiotic against *Pseudomonas aeruginosa* isolated from wounds. Antibiotics gentamicin and nitrofurantoin remains the drug of choice for UTI patients while other antibiotics such as tetracycline and penicillin have turned ineffective.

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