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Prevalence and Antimicrobial susceptibility pattern of Gram negative bacteria of postoperative wounds in hospitals of Omerga Region, Maharashtra, India

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Postoperative wound, Prevalence, Antibiotic, Antimicrobial Resistance

A B S T R A C T

The study was aimed to determine the prevalence of aerobic nosocomial Gram negative bacteria among patients with postoperative wound infections and their antimicrobial susceptibility pattern. This study was conducted for a period of 28 months from September 2011 to December 2013. A total of 83 patients with clinically suspected post-operative wound infections were enrolled in the study. Conventional microbiological techniques were used for isolation and identification of bacteria. Antimicrobial susceptibility testing was performed to all pathogenic isolates using Kirby-Bauer disc diffusion method according to the CLSI guidelines 2009. In respect of post operative wound discharge and incriminated organisms, it was found that most of the surgical site infections were due to *Escherichia coli* (20.5%), *Klebsiella pneumoniae* (14.45%), *Acinetobacter baumannii (13.25%) and Pseudomonas aeruginosa* (12.08 %). A high level of AMR was observed in gram negative bacterial isolates. Rational use of antibiotics and a regular monitoring of antimicrobial resistance patterns in post-operative wound infections are essential and mandatory to prevent further emergence and spread of anti-microbial resistance among bacterial pathogens.

Introduction

Postoperative infections have been found to pose a major problem in the field of surgery for a long time. It is also called surgical site infection (SSI). Uncontrolled and rapidly spreading anti-microbial resistance among bacterial populations has made the management and treatment of post-operative wound infections a serious challenge in clinical and surgical practice (Adegoke *et al.*, 2010).

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Prolonged courses of antibiotics and their combinations, some of which begin empirically results in the selection of multidrug resistance nosocomial Gram negative bacteria mainly *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* species (Agwunglefah *et al.*, 2014). Enteric group of organisms tend to be endemic in hospital environment by being easily transferred from object to object, they also tend to be resistant to common antiseptics and are difficult to eradicate in the long term and these group of organisms are increasingly playing a greater role in the many hospital acquired infections (Amrita *et al.*, 2010, Ananth and Rajan, 2014). *Enterococci* are also posing major problems with resistance; glycopeptide-resistant *Enterococci* are now found in many hospitals and they may cause life-threatening infections in immunocompromised patients. Gram-negative organisms such as *Pseudomonas aeruginosa* may also be multiresistant. The increasing use of third-generation Cephalosporins appears to be encouraging the emergence of Gram-negative bacilli such as *Klebsiella pneumoniae* and *Enterobacter cloacae* resistant to these and other β-lactams (Jadhav *et al.*, 2012).

The first incidence of antibiotic resistance to penicillin soon brought novel challenges in the treatment of infection. Although the development of new antibiotics has occurred at an extraordinary place in recent years, it was paralleled by the appearance of resistance to antibiotics (Leung-Kei, 2002).

Surveillance, which records infection prospectively and actively, is an essential method for understanding the incidence and distribution of healthcare-associated infections (Nabakishore, 2014). Site-oriented target surveillance, which is usually undertaken for selected high-risk infections and specialties, provides more accurate data. Periodic surveillance of the species of bacteria involved in post-operative wound infection and determination of their antimicrobial resistance is recommended for empirical treatment (Guta *et al.*, 2014).

Materials and Methods

The study was conducted in the microbiology laboratory, Adarsh Mahavidyalaya, Omerga, Maharashtra, India. All the specimens received from patients hospitalized from September 2011 to December 2013 were processed for isolation and identification of bacterial pathogens, according to the standard microbiological techniques. A total of 83 postoperative wound swabs were collected aseptically with a sterile cotton wool swab from clinically suspected infected wounds from different wards. Gram stain preparations were made from all swabs. Samples were inoculated onto 5% sheep blood agar, MacConkey agar. The plates were incubated at 37°C for 18–24 hours. The cultures were read after 24 hours but extended to 48 hours if there was no bacterial growth after 24 hours. Isolated organisms presented to Gram stain and biochemical tests for identification. Identification was carried out according to the standard biochemical tests. Antimicrobial susceptibility testing was performed on Muller-Hinton agar using Kirby-Bauer disc diffusion method according to the CLSI guidelines.

Clinical specimens

Specimens were collected aseptically with sterile cotton wool swabs from post operative wound infections. Pus samples / wound swabs were collected with aseptic precautions and were transported to the laboratory without delay.

Culture media and biochemical tests

Blood agar, MacConkey agar and Nutrient agar were used for isolation and study of cultural characters. The plates were incubated at 37°C for 24 hours in an incubator. Isolated colonies were subjected to Gram staining and biochemical tests for identification. Biochemical tests are performed by API20E and Vitek2 systems. Most resistant isolate is further identified by 16S rRNA sequencing.

Antibiotic susceptibility testing

Antimicrobial susceptibility test were carried out on isolated and identified colonies of Gram-negative bacteria using commercially prepared antibiotic disk (Span diagnostics) on Nutrient agar plates by the disk diffusion method, according to the Central Laboratory Standards Institute (CLSI) guidelines. Antibiotics used in our study were Ticarcillin / Clavulanic acid, Meropenem, Levofloxacin, Moxifloxacin, Cefprozil, Cefirome, Ceftizoxime, Cefpodoxime, Cefoperazone / Sulbactam, Sparfloxacin, Pipercillin / Tazobactum, Gatifloxacin, Imipenem / Cilastatin and Tobramycin.

Result and Discussion

Table 1 shows Zone of inhibition (in mm) of Gram negative isolates to different antibiotics. Table 2 shows Antimicrobial Resistance pattern of Gram negative bacterial isolates.

A total of 83 specimens were obtained from postoperative wounds, including superficial and deep-seated infections of all patients hospitalized at surgical, pediatrics, orthopedic, obstetrics, and gynecology wards. Out of 83 specimens 63 (76%) were Gram negative bacteria. The majority of Gram negative bacterial isolates were resistant to Cefprozil (94.9%), Moxifloxacin (96.6%), Ticarcillin/Clavulanic acid (86.4%), Cefpodoxime (91.5%), Sparfloxacin (91.5%), Gatifloxacin (98.3%), Ceftazidime (80%), Cefotaxime (100%) and Ceftrixone (84.8%) See table no. 3.

The most common isolated Gram negative bacteria from postoperative wounds were *Escherichia coli* (20.5%), *Klebsiella pneumoniae* (14.45%), *Acinetobacter baumannii (13.25%) and Pseudomonas aeruginosa* (12.08 %).

Antimicrobial Resistance patterns of Gram negative bacteria

The majority of Gram negative bacterial isolates were sensitive to Colistin, Amikacin, Meropenem, Cefoperazone/ Sulbactam, Tigecycline. Thus, these drugs appear to be effective against post surgical wound infection in the study area. These antibiotics should however be used with caution because of the emerging low level of resistance which may indicate great danger for their future use. The majority of Gram negative bacterial isolates were resistant to Cefprozil (94.9%), Moxifloxacin (96.6%), Ticarcillin/Clavulanic acid (86.4%), Cefpodoxime (91.5%), Sparfloxacin (91.5%), Gatifloxacin (98.3%), Ceftazidime (80%), Cefotaxime (100%) and Ceftrixone (84.8%) (Akingbade *et al.*, 2012).

A hospital based cross-sectional study by Lopiso *et al.* (2014) in Ethiopia showed that, out of total 177 aerobic bacteria isolates; 105 (59.3 %) were Gram-negative and 72 (40.7%) were Gram-positive organisms. In this study, multiple antibiotics resistance was seen (64.55%) in the Gram negative isolates. This is in agreement with previous studies (Biadglegne *et al.*, 2009; Mulu *et al.*, 2006).

Table.1 Zone of inhibition (in mm) of Gram negative isolates to different antibiotics

Name of isolate	C. No	TC	MR	LV	MF	FP	CE	FO	CO	CS	PT	SP	GF	IS	TO
Acinetobacter baumannii	**12**	14	16	–	–	10	14	16	14	14	14	–	–	18	14
	22	7	15	–	–	11	15	15	14	10	9	–	–	10	10
	70	–	–	–	–	–	7	–	–	22	–	–	–	15	–
	76	19	–	–	–	15	–	–	–	18	20	–	–	–	–
	77	–	–	–	–	–	–	7	16	14	10	–	12	14	–
	81	–	12	–	–	–	–	–	–	14	16	–	–	–	12
Acinetobacter baumannii complex	**8**	–	–	14	–	–	12	12	12	12	10	8	–	–	14
	13	–	24	–	–	–	–	10	–	14	12	–	–	18	–
	24	–	–	–	–	–	–	–	–	18	8	–	–	–	12
	27	–	10	–	–	–	10	12	11	16	9			10	13
	43	–	18	20	–	–	10	11	10	–	18	–	–	15	16
Citrobacter koseri	**35**	–	19	18	10	–	17	8	10	9	19	–	11	16	15
	57	–	–	17	11	7	18	7	10	10	20	–	13	15	17
Enterobacter cloacae sp cloacae	**71**	18	16	14	22	20	10	–	–	20	17	20	22	8	18
Enterobacter cloacae sp dissolvens	**39**	17	15	13	21	20	11	–	–	19	13	18	20	9	13
Escherichia coli	**4**	11	18	7	–	–	13	18	–	15	15	–	9	15	14
	14	14	20	–	–	–	12	16	–	16	14	–	–	18	12
	16	12	19	–	–	–	13	17	–	14	16	–	–	14	15
	17	10	20		–	–	12	20	–	12	15	–	–	14	10
	18	11	17	–	–	–	13	18	8	19	14	–	10	15	16
	25	12	15	8	–	–	12	–	–	17	–	16	10	8	8
	29	10	18	9	7	–	11	–	–	–	15	–	–	16	15
	31	12	20	7	–	–	12	–	–	16	13	–	10	12	10
	33	11	20	14	8	–	10	17	–	16	16	–	11	15	16
	34	11	17	9	–	–	11	16	9	15	15	12	10	16	15
	37	12	18	–	7	–	10	16	–	19	14	10	12	16	12
	41	11	19	7	8	–	12	15	–	18	17	11	10	16	15
	45	12	17	8	7	–	10	16	8	20	18	–	12	15	16
	51	11	21	–	–	–	11	17	–	17	18	10	11	15	16
	52	13	28	8	6	–	10	20	10	30	15	–	12	18	24
	54	13	10	13	12	–	20	14	–	20	16	13	13	14	16
	75	–	20	–	–	–	–	10	–	20	12	–	–	14	–
Klebsiella pneumoniae sp pneumoniae	**30**	12	18	–	–	–	16	20	–	20	16	–	8	18	10
Klebsiella pneumoniae	**1**	–	20	12	12	–	10	12	–	20	10	12	–	–	–
	2	8	24	8	–	–	12	–	–	24	18	12	8	20	22

	7	9	21	10	–	–	11	13	–	18	15	10	–	19	10
	11	12	16	8	–	20	14	16	14	16	12	–	–	16	12
	15	10	17	9	–	–	12	13	10	17	15	9	–	17	16
	26	–	16	–	–	–	–	–	–	14	14	–	–	16	10
	40	11	19	11	–	9	13	15	11	17	18	10	–	17	14
	42	10	12	9	11	–	14	14	9	16	10	15	11	12	11
	44	16	18	14	–	–	10	–	–	17	11	10	–	16	17
	50	17	11	15	14	–	11	–	–	15	10	–	9	11	10
	53	20	10	–	–	–	–	–	–	13	18	–	–	16	16
Morganella morganii sub sp. morganii	**55**	–	18	7	–	–	10	10	–	11	12	–	–	–	10
Proteus penneri	**62**	10	20	17	7	–	14	17	–	16	16	7	–	7	7
	64	11	18	18	–	8	13	15	–	15	17	6	8	–	9
Pseudomons aeruginosa	**3**	–	17	14	–	–	–	–	–	–	16	–	–	17	16
	5	–	19	10	–	–	–	–	–	–	17	–	–	16	10
	6	–	8	–	–	–	10	14	14	12	10	–	–	16	–
	38	–	14	17	–	–		14	–	10	15	–	–	10	16
	46	–	17	9	–	–	9	–	–	16	14	–	–	15	17
	68	–	11	–	–	–	–	–	–	18	–	11	–	–	–
	69	15	12	14	7	–	–	–	–	20	12	15	8	–	16
	78	9	14	10	–			15		19	10			10	11
	80	7	10	9	–	–	12	15	–	16	11	–	–	9	10
	82	10	15	11	–	–	10	16	–	15	14	–	–	11	11
Salmonella typhi	**56**	12	15	16	11	12	14	20	–	11	18	11	12	15	12
Serratia fonticola	**79**	–	17	–	–	–	–	–	–	16	15	–	–	–	15
Serratia liquefacians	**61**	14	6	18	8	18	7	7	–	10	12	16	24	7	10

Sensitivity and Resistance to different antibiotics is determined by referring zone diameter interpretive chart (as per CLSI JANUARY 2007) (M100 S17, Vol.27 No.1) (Replaces M100 S16, Vol.26, 0. 3)

TC– Ticarcillin / Clavulanic acid
IS– Imipenem / Cilastatin
CE – Cefirome
CS– Cefoperazone / Sulbactam
PT – Pipercillin / Tazobactum
MR – Meropenem
MF– Moxifloxacin
FO – Ceftizoxime
SP – Sparfloxacin
TO – Tobramycin
LV – Levofloxacin
FP – Cefprozil
CO – Cefpodoxime
GF – Gatifloxacin

Table.2 Antimicrobial resistance pattern of Gram negative bacterial isolates

Name of isolate	Cul.No	TC	MR	LV	MF	FP	CE	FO	CO	CS	PT	SP	GF	IS	TO
Acinetobacter baumannii	**12**	R	S	R	R	R	S	S	S	S	S	R	R	S	S
	22	R	S	R	R	R	S	S	S	R	R	R	R	R	R
	70	R	R	R	R	R	R	R	R	S	R	R	R	S	R
	76	S	R	R	R	S	R	R	R	S	S	R	R	R	R
	77	R	R	R	R	R	R	R	S	S	R	R	R	I	R
	81	R	R	R	R	R	R	R	R	I	S	R	R	R	R
Acinetobacter baumannii complex	**8**	R	S	I	R	R	R	R	R	R	R	R	R	R	S
	13	R	S	R	R	R	R	R	R	I	R	R	R	S	R
	24	S	R	R	R	R	R	R	R	S	R	R	R	R	S
	27	R	R	R	R	R	R	R	R	S	R	R	R	R	I
	43	R	S	S	R	R	R	R	R	R	S	R	R	S	S
Citrobacter koseri	**35**	R	S	S	R	R	S	R	R	R	S	R	R	S	S
	57	R	R	S	R	R	S	R	R	R	S	R	R	S	S
Enterobacter cloacae sp cloacae	**71**	S	R	R	S	S	R	R	R	S	R	S	S	R	S
Enterobacter cloacae sp dissolvens	**39**	S	R	R	S	S	R	R	R	S	R	S	S	R	R
Escherichia coli	**4**	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	14	R	S	R	R	R	R	S	S	R	I	R	R	S	R
	16	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	17	R	S	R	R	R	R	S	R	R	S	R	R	S	R
	18	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	25	R	S	R	R	R	R	R	R	S	R	S	R	R	R
	29	R	S	R	R	R	R	R	R	R	S	R	R	S	S
	31	R	S	R	R	R	R	R	R	S	R	R	R	S	S
	33	R	S	S	R	R	R	S	R	S	S	R	R	S	S
	34	R	S	R	R	R	R	R	R	R	S	R	R	S	S
	37	R	S	R	R	R	R	S	R	S	I	R	R	S	R
	41	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	45	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	51	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	52	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	54	R	S	R	R	R	R	S	R	S	S	R	R	S	S
	75	R	S	R	R	R	R	R	R	S	R	R	R	S	R

Klebsiella pneumoniae sp pneumoniae	**30**	R	S	R	R	R	S	S	R	S	S	R	R	S	R
Klebsiella pneumoniae	**1**	R	S	R	R	R	R	R	R	S	R	R	R	R	R
	2	R	S	R	R	R	R	R	R	S	S	R	R	S	S
	7	R	S	R	R	R	R	R	R	S	R	R	R	S	R
	11	R	S	R	R	R	S	I	R	S	R	R	R	S	R
	15	R	S	R	R	R	R	R	R	S	S	R	R	S	S
	26	R	S	R	R	R	R	R	R	S	R	R	R	S	R
	40	R	S	R	R	R	I	S	R	S	S	R	R	S	I
	42	R	R	R	R	R	S	S	R	S	R	S	S	R	R
	44	S	S	I	R	R	R	R	R	S	R	R	R	S	S
	50	S	R	S	I	R	R	R	R	S	R	R	R	R	R
	53	S	R	R	R	R	R	R	R	I	S	R	R	S	S
Morganella morganii sub sp. morganii	**55**	R	S	R	R	R	R	R	R	R	R	R	R	R	R
Proteus penneri	**62**	R	S	S	R	R	I	S	R	S	S	R	R	R	R
	64	R	S	S	R	R	I	S	R	S	S	R	R	R	R
Pseudomonas aeruginosa	**3**	R	S	I	R	R	R	R	R	S	S	R	R	S	S
	5	R	S	R	R	R	R	R	R	R	S	R	R	S	R
	6	R	R	R	R	R	R	S	S	R	R	R	R	S	R
	38	R	I	S	R	R	R	S	R	R	S	R	R	R	S
	46	R	S	R	R	R	R	R	R	S	I	R	R	S	S
	68	R	R	R	R	R	R	R	R	S	R	R	R	R	R
	69	S	R	I	R	R	R	R	R	S	R	S	R	R	S
	78	R	I	R	R	R	R	S	R	S	R	R	R	R	R
	80	R	R	R	R	R	R	S	R	S	I	R	R	R	R
	82	R	S	R	R	R	R	S	R	S	I	R	R	R	R
Salmonella typhi	**56**	R	S	S	R	R	I	S	R	R	S	R	R	S	R
Serratia fonticola	**79**	R	S	R	R	R	R	R	R	S	S	R	R	R	S
Serratia liquefacians	**61**	I	R	S	R	S	R	R	R	R	R	S	S	R	R

Table.3 Percentage of antimicrobial resistance of Gram negative bacterial isolates

Antibiotics	%Resist-ance	Antibiotics	%Resi-stance	Antibiotics	%Resi-stance
Ticarcillin / Clavulanic acid	86.4	Imipenem / Cilastatin	38.9	Trimethoprim/ sulfamethoxazole	59.3
Meropenem	27.1	Gatifloxacin	98.3	Gentamycin	52.5
Levofloxacin	76.2	Sparfloxacin	91.5	Ciprofloxacin	67.7
Moxifloxacin	96.6	Tobramycin	50.8	Tetracycline	52.3
Cefprozil	94.9	Ampicillin /Sulbactum	77.7	Pipercillin / Tazobactum	40.6
Cefirome	79.6	Ceftazidime	80	Colistin	16.1
Ceftizoxime	55.9	Cefotaxime	100	Amikacin	27.1
Cefpodoxime	91.5	Ceftrixone	84.8	Tigecycline	31

However, the high frequency of multiple antibiotics resistance might be a reflection of inappropriate use of antimicrobials, lack of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics. Multiple antibiotics resistance to these commonly used antibiotics is found to be extremely high which makes the condition frustrating. Most of the isolates were resistant to these antibiotics. This finding is relatively higher as compared to other studies (Mulu *et al.*, 2006, Biadglegne *et al.*, 2009). This may be explained by the fact that, irrational use of antibiotics for conditions that may not clinically indicate their use, over the counter sell of antibiotics, some new drug formulations which may be of poor quality and dumping of banned products into the market where the public may get access to them hence antimicrobial resistance strains grow around.

In the present study Gram negative bacteria displayed high rates of resistance to common prescribed antibiotics such as Cefotaxime (100%), Ceftrixone (84.8%), Ticarcillin/Clavulanic acid (86.4%), Moxifloxacin (96.6%), Cefprozil (94.9%), Sparfloxacin (91.5%), Gatifloxacin (98.3%) and Ceftazidime (80%). These results are in agreement with the study of Apisarnthanarak *et al.* (2007), where in, Gram negative bacteria displayed high rates of resistance to common prescribed antibiotics such as Cefotaxime (100%), Gatifloxacin (98.3%), Cefprozil (94.9%), Moxifloxacin (96.6%), Ticarcillin / Clavulanic acid (86.4%), Cefpodoxime (91.5%) and Sparfloxacin (91.5%). Therefore use of these drugs in treatment of surgical site infections should be closely monitored for clinical response and be guided by microbiological testing. Injudicious use of this antibiotic at this tertiary facility probably can explain the increasing trend of resistance, as unpublished data suggest it's among most prescribed antibiotic at the hospital. This data suggest that β–lactam / β–lactamase inhibitor combination may not be useful for empirical treatment of Gram negative bacteria SSI in our setting.

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