ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF AEROBIC BACTERIAL ISOLATES CAUSING WOUND INFECTIONS AMONG THE PATIENTS ATTENDING TO SANTHIRAM GENERAL HOSPITAL, NANDYAL

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ABSTRACT

BACKGROUND

The increasing trends in the rate of the antibiotic resistance have now become a serious and an increasingly common concern with severe implications, especially in the surgical departments. These multidrug resistance patterns which include ESBLs, AmpC β -lactamases and metallo- β -lactamases have emerged as the most worrisome mechanisms of resistance among the gram-negative bacteria, which pose a therapeutic challenge to the healthcare settings.

MATERIALS AND METHODS

This descriptive study was undertaken to evaluate the profile of aerobic pyogenic bacteria in various pus isolates along with the changing trends in antimicrobial resistance in a tertiary hospital in Nandyal from January 2017 to December 2017. A total of 283 bacterial isolates from 577 clinical samples, which were received over a period of one year were processed. After their identification and their antimicrobial susceptibility testing, they were then screened for the multidrug resistance.

RESULTS

Among the 283 isolates, most predominant organism was *Escherichia coli* 63 (22.18%) followed by *Pseudomonas aeruginosa* 60 (21.13%) and *Staphylococcus aureus* 58 (20.42%). The antibiogram of gram-positive cocci revealed that they were most susceptible to Vancomycin (100%) and Linezolid (100%). Gram-negative Bacilli are susceptible to Imipenem (96.23%), Cefoperazone + Sulbactam (77.22%) and Netilmicin (75.47%). Of the 212 gram-negative isolates, 143 (67.45%) were ESBL producers followed by 36 (16.98%) AmpC producers and 7 (3.30%) metallo- β -lactamase (MBL) producers. The major ESBL and AmpC producer was *Klebsiella pneumoniae*, while the MBL production was mainly observed in *Pseudomonas aeruginosa*.

CONCLUSION

The high prevalence of the multidrug resistant isolates emphasises the need for a continuous surveillance in the healthcare settings to detect the resistant strains, strict guidelines for the antibiotic therapy and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance.

KEY WORDS

Pus, Pyogenic, Antibiotic Susceptibility Pattern, Multidrug Resistance, MBL, ESBL, AmpC.

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BACKGROUND

Pyogenic infection is generally caused by one of the pyogenic bacteria and characterised by severe local inflammation, usually with pus formation. Pyogenic infections can be either endogenous or exogenous. A discontinuity in the skin can provide an entryway for these surface bacteria into the body and the bacteria multiply and extend into the cut.

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The body's defence mechanism brings immune cells into the area to fight against the bacteria. As a result, accumulation of these cells produces the thick whitish pus. With the increase of antibiotic use in the recent years, the emergence of resistance of the bacteria is also at rise. Various studies have been consistent enough to show a predictable bacterial profile and their resistance patterns in the pyogenic wound infections. This helps a clinician who intends to start empirical treatment to his patients, while laboratory culture reports are awaited.

This study was undertaken to evaluate the profile of aerobic pyogenic bacteria in various pus isolates along with the changing trends in antimicrobial resistance in a tertiary hospital in Nandyal from January 2017 to December 2017.

MATERIALS AND METHODS

This was a descriptive study. A total number of 577 pus samples received for aerobic culture and sensitivity from

IPDs and OPDs of various departments to Microbiology Central Laboratory of Santhiram General Hospital, Nandyal during a period from January 2017 to December 2017 were included in the study.

The samples were inoculated on Blood Agar, Chocolate Agar, MacConkey's Agar and Nutrient Agar media as soon as they were received and incubated at 37°C for 24 hours under aerobic condition in incubator, and under sterile conditions. If there was no growth within 24 hours, the plates were further incubated for 24 hours. If growth was seen, the bacterial species were identified on basis of colony morphology, morphology of bacteria, staining characters, culture characteristics and biochemical reactions according to standard techniques. For testing the antibiotic sensitivity, Kirby Bauer's Disk Diffusion method was used and the isolates were inoculated uniformly on Muller-Hinton agar plates and the antibiotic discs were placed firmly on the surface of the medium with sterile forceps. After incubation at 37°C for 18 - 24 hours, the zone of inhibition was noted.^[1,2] Results were interpreted comparing with the standard zone size as per Clinical Laboratory Standard Institution (CLSI) guidelines.^[3]

Standard antibiotics like Amikacin (30 mcg), Amoxyclav (20/10 mcg), Ampicillin (10 mcg), Aztreonam (30 mcg), Ceftazidime Clavulanic Acid (30/10 mcg), Ceftazidime (30mcg), Clindamycin (2 mcg), Cefoperazone + Sulbactam (75/10 mcg), Ciprofloxacin (5 mcg), Ceftriaxone (30 mcg), Cefotaxime (5 mcg), Cefoxitin (30 mcg), Cefazolin (30 mcg), Doxycycline (30 mcg), Erythromycin (15 mcg), Gentamycin (10 mcg), Imipenem (10 mcg), Linezolid (30 mcg), Netilmicin (30 mcg), Penicillin-G (10 units), Piperacillin (100 mcg), Piperacillin + Tazobactam (30/6 mcg), Sparfloxacin (5 mcg), Teicoplanin (30 mcg) and Vancomycin (30 mcg)^[4] were tested. All antibiotic discs were procured from HiMedia, Mumbai, India.

Isolates resistant to third generation cephalosporins were tested for ESBL production and isolates showing resistance to imipenem were tested for MBL production. Phenotypic detection of ESBL was done by the double disk diffusion method using ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/10 µg). The strains were screened for the AmpC β-lactamase production by the disc antagonism test. Imipenem resistant isolates were screened for production of MBL by double disc diffusion test using commercially available imipenem (10 µg) and imipenem/EDTA (10 + 750 µg).

RESULTS

Out of total 577 pus samples received, 491 (85.1%) were from inpatients and 86 (14.90%) were from outpatient departments [Figure 1].

Figure 1 : Distribution of samples

265 (45.93%) samples were culture positive and 312 (54.07%) were culture negative [Figure 2].

265 culture positive samples yielded 283 isolates. 93% (246) showed monomicrobial growth and 7% (19) showed mixed growth [Figure 3].

Figure 3 : Distribution of isolates among the pus samples

Out of 283 samples, seventy one (25.09%) of the isolates were gram positive and 212 (74.91%) were gram negative [Figure 4].

Figure 4 : Distribution of isolates

Among 265 culture positive samples, 182 (68.68%) were from male patients and 83 (31.32%) were from female patients [Figure 5].

Figure 5 : Gender wise distribution of patients with culture positive

Majority of the cultures were positive from 51 - 60 years' age group comprising of 22% (59) [Figure 6].

The department-wise distribution of pus samples revealed that surgery dept. was the highest contributors (72.79%) followed by Orthopaedics (12.82%), Gynaecology and Obstetrics (5.55%), Medicine (5.37%), Paediatrics (1.21%), TB and CD (1.21%), DVL (0.69%) and ENT (0.35%) departments [Table 1].

Department	Total Samples	Culture Positive
Surgery	402 (69.67%)	180 (44.78%)
Orthopaedics	74 (12.82%)	41 (55.41%)
ICU	34 (5.89%)	12 (35.29%)
OBG	32 (5.55%)	20 (62.50%)
Medicine	15 (2.60%)	4 (26.67%)
Paediatrics	7 (1.21%)	2 (28.57%)
TB and CD	7 (1.21%)	4 (57.14%)
DVL	4 (0.69%)	1 (25.00%)
ENT	2 (0.35%)	1 (50.00%)
Total	577 (100.00%)	265 (45.58%)

Table 1. Department-Wise distribution of Samples and Culture Positivity

Organism-wise distribution of positive cultures showed highest isolation of *Escherichia coli* followed by *Pseudomonas*, *Staphylococcus* and *Klebsiella*. Among the gram-positive bacteria isolated, most predominant organism was *Staphylococcus aureus* 58 (20.42%) followed by *Streptococcus pyogenes* 8 (2.82%), *Enterococcus* species 3 (1.06%) and CONS (*Staphylococcus epidermidis*) 2 (0.70%). Among the gram-positive bacteria isolated, most predominant organism was *Escherichia coli* 63 (22.18%) followed by *Pseudomonas aeruginosa* 60 (21.13%), *Klebsiella pneumoniae* 57 (20.07%) and *Proteus* sp. 16 (5.63%), *Citrobacter* 15 (5.28%) and *Enterobacter* 1 (0.35%). One fungal isolate was identified as *Candida* spp. (0.35%) [Table-2].

Organisms Distribution	Number
<i>Escherichia coli</i>	63 (22.18%)
<i>Pseudomonas aeruginosa</i>	60 (21.13%)
<i>Staphylococcus aureus</i>	58 (20.42%)
<i>Klebsiella pneumoniae</i>	57 (20.07%)
<i>Proteus</i> species	16 (5.63%)

Name of Antibiotics	<i>Escherichia coli</i> (n=63)		<i>Klebsiella pneumoniae</i> (n=57)		<i>Proteus</i> SPP (n=16)		<i>Citrobacter</i> Spp. (n=15)		<i>Enterobacter</i> (n=1)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Amikacin	90.48%	9.52%	61.40%	38.60%	56.25%	43.75%	60.00%	40.00%	100.00%	0.00%
Amoxycylav	11.11%	88.89%	5.26%	94.74%	12.50%	87.50%	6.67%	93.33%	0.00%	100.00%
Ampicillin	7.94%	92.06%	3.51%	96.49%	12.50%	87.50%	6.67%	93.33%	0.00%	100.00%
Ceftazidime	36.51%	63.49%	15.79%	84.21%	31.25%	68.75%	20.00%	80.00%	0.00%	100.00%
Cefoperazone+Sulbactam	79.37%	20.63%	64.91%	35.09%	87.50%	12.50%	93.33%	6.67%	100.00%	0.00%
Ciprofloxacin	39.68%	58.73%	31.58%	68.42%	75.00%	25.00%	60.00%	40.00%	100.00%	0.00%

<i>Citrobacter</i> species	15 (5.28%)
<i>Streptococcus pyogenes</i>	8 (2.82%)
<i>Enterococcus</i> species	3 (1.06%)
Cons	2 (0.70%)
<i>Enterobacter</i>	1 (0.35%)
<i>Candida</i>	1 (0.35%)
Total	284 (100.00%)

Table 2. Organism-Wise distribution of Culture Positivity

The Antibigram of gram-positive cocci revealed that most isolates were susceptible to Vancomycin (100%) and linezolid (100%) [Table-3] [Table-4].

Name of Antibiotics	<i>Staphylococcus aureus</i> (n= 58)		CONS (n= 2)	
	Sensitive	Resistant	Sensitive	Resistant
Amoxyclav	46.55%	53.45%	0.00%	100.00%
Clindamycin	82.76%	17.24%	50.00%	50.00%
Ciprofloxacin	82.76%	17.24%	50.00%	50.00%
Cefoxitin	98.28%	1.72%	100.00%	0.00%
Cefazolin	89.66%	10.34%	50.00%	50.00%
Erythromycin	56.90%	43.10%	50.00%	50.00%
Gentamycin	87.93%	12.07%	50.00%	50.00%
Linezolid	100.00%	0.00%	100.00%	0.00%
Netilmicin	91.38%	8.62%	100.00%	0.00%
Penicillin-G	10.34%	89.66%	0.00%	100.00%
Sparfloxacin	94.83%	5.17%	100.00%	0.00%
Teicoplanin	100.00%	0.00%	100.00%	0.00%
Vancomycin	100.00%	0.00%	100.00%	0.00%

Table 3. Antibigram of Gram-Positive Cocci (*Staphylococcus* species)

Name of Antibiotics	<i>Streptococcus Pyogenes</i> (n= 8)		<i>Enterococcus</i> (n= 3)	
	Sensitive	Resistant	Sensitive	Resistant
Amoxyclav	75.00%	25.00%	33.33%	66.67%
Ampicillin	75.00%	25.00%	33.33%	66.67%
Aztreonam	100.00%	0.00%	33.33%	66.67%
Clindamycin	50.00%	50.00%	33.33%	66.67%
Ciprofloxacin	50.00%	50.00%	100.00%	0.00%
Cefazolin	50.00%	50.00%	100.00%	0.00%
Doxycycline	100.00%	0.00%	100.00%	0.00%
Erythromycin	37.50%	62.50%	100.00%	0.00%
Gentamycin	50.00%	50.00%	100.00%	0.00%
Linezolid	100.00%	0.00%	66.67%	33.33%
Penicillin-G	25.00%	75.00%	0.00%	100.00%
Teicoplanin	100.00%	0.00%	66.67%	33.33%
Vancomycin	100.00%	0.00%	100.00%	0.00%

Table 4. Antibigram of Gram-Positive Cocci (*Streptococcus* and *Enterococcus* species)

Ceftriaxone	34.92%	65.08%	15.79%	84.21%	43.75%	56.25%	20.00%	80.00%	0.00%	100.00%
Gentamycin	85.71%	14.29%	56.14%	43.86%	62.50%	37.50%	66.67%	33.33%	100.00%	0.00%
Imipenem	98.41%	1.59%	96.49%	3.51%	93.75%	6.25%	100.00%	0.00%	100.00%	0.00%
Netilmicin	87.30%	12.70%	64.91%	35.09%	56.25%	43.75%	73.33%	26.67%	100.00%	0.00%
Piperacillin	26.98%	73.02%	19.30%	80.70%	62.50%	37.50%	40.00%	60.00%	0.00%	100.00%
Piperacillin+ Tazobactam	73.02%	26.98%	57.89%	42.11%	87.50%	12.50%	73.33%	26.67%	100.00%	0.00%
Sparfloxacin	46.03%	53.97%	52.63%	47.37%	62.50%	37.50%	86.67%	13.33%	100.00%	0.00%

Table 5. Antibigram of Enterobacteriaceae members

Gram-negative Bacilli are susceptible to Imipenem (96.23%), Cefoperazone + Sulbactam (77.22%), Netilmicin (75.47%), Amikacin (75.00%) [Table-5] [Table-6].

Name of Antibiotics	Pseudomonas Spp.	
	Sensitive	Resistant
Amikacin	80.00%	20.00%
Ciprofloxacin	80.00%	20.00%
Cefotaxime	40.00%	60.00%
Gentamycin	75.00%	25.00%
Imipenem	93.33%	6.67%
Netilmicin	78.33%	21.67%
Piperacillin	70.00%	30.00%
Piperacillin + Tazobactam	83.33%	16.67%

Table 6. Antibigram of Non-Fermenters

Of the 212 gram-negative isolates, 143 (67.45%) were ESBL producers followed by 36 (16.98%) AmpC producers and 7 (3.30%) metallo- β -lactamase (MBL) producers. The Major ESBL producer was *Klebsiella pneumoniae* (32.87%) followed by *Escherichia coli* (27.27%) and *Pseudomonas aeruginosa* (25.17%). The AmpC production was also maximally seen in *Klebsiella pneumoniae* (55.56%), while the MBL production was mainly observed in *Pseudomonas aeruginosa* (57.14%) [Table-7].

Organisms	ESBL		AMPC		MBL	
	No.	%	No.	%	No.	%
<i>Escherichia coli</i>	39	27.27%	13	36.11%	1	14.29%
<i>Pseudomonas aeruginosa</i>	36	25.17%	0	0.00%	4	57.14%
<i>Klebsiella pneumoniae</i>	47	32.87%	20	55.56%	1	14.29%
Proteus species	8	5.59%	2	5.56%	1	14.29%
Citrobacter species	12	8.39%	1	2.78%	0	0.00%
Enterobacter	1	0.70%	0	0.00%	0	0.00%
Total	143	100.00%	36	100.00%	7	100.00%

Table 7. Organism-Wise distribution of Multidrug Resistant Isolates

Highest number of MDR isolates were recovered from samples received from surgery department (65.03%) followed by Orthopaedics (13.99%) and Gynaecology and Obstetrics (12.59%) [Table-8].

	ESBL (n= 143)	AMPC (n= 36)	MBL (n= 7)
Surgery	93 (65.03%)	26 (72.22%)	4 (57.14%)
Orthopaedics	20 (13.99%)	4 (11.11%)	3 (42.86%)
Gynaecology and Obstetrics	18 (12.59%)	2 (5.56%)	0 (0.00%)
ICU	8 (5.59%)	3 (8.33%)	0 (0.00%)
Medicine	2 (1.40%)	1 (2.78%)	0 (0.00%)
Paediatrics	0 (0.00%)	0 (0.00%)	0 (0.00%)
TB and CD	1 (0.70%)	0 (0.00%)	0 (0.00%)
DVL	1 (0.70%)	0 (0.00%)	0 (0.00%)
ENT	0 (0.00%)	0 (0.00%)	0 (0.00%)
Total	143 (100.00%)	36 (100.00%)	7 (100.00%)

Table 8. Department-Wise distribution of Multidrug Resistant Isolates

DISCUSSION

The present study revealed that among the gram-positive bacteria isolated, most predominant organism was *Staphylococcus aureus* 58 (20.42%) followed by *Streptococcus pyogenes* 8 (2.82%), enterococcus species 3 (1.06%) and CONS (*Staphylococcus epidermidis*) 2 (0.70%). Among the gram-positive bacteria isolated, most predominant organism was *Escherichia coli* 63 (22.18%) followed by *Pseudomonas aeruginosa* 60 (21.13%), *Klebsiella pneumoniae* 57 (20.07%) and *Proteus* sp. 16 (5.63%) *Citrobacter* 15 (5.28%) and enterobacter 1 (0.35%). One fungal isolate was identified as *Candida* spp (0.35%). Dominance of gram-negative bacteria as the causative agent of pyogenic lesions is seen which is supported by Zubair et al,^[5] Agnihotri N et al,^[6] Ghosh A et al^[7] and Basu S et al.^[8]

The department wise distribution of pus samples revealed that surgery dept. was the highest contributors (72.79%) followed by Orthopaedics (12.82%), Gynaecology and Obstetrics (5.55%), Medicine (5.37%), Paediatrics (1.21%), TB and CD (1.21%), DVL (0.69%) and ENT (0.35%) departments.

The Antibigram of *Staphylococcus aureus* revealed that the Vancomycin (100%), teicoplanin (100%) and linezolid (100%) were the most susceptible drugs. The Antibigram of coagulase negative staphylococci revealed that the Vancomycin (100%), teicoplanin (100%), linezolid (100%), sparfloxacin (100%), netilmicin (100%) and cefoxitin (100%) were the most susceptible drugs. The Antibigram of streptococci revealed that the Vancomycin (100%), Teicoplanin (100%), Linezolid (100%), Doxycycline (100%) and Aztreonam (100%) were the most susceptible drugs. The Antibigram of Enterococci revealed that the Vancomycin (100%), Ciprofloxacin (100%), Cefazolin (100%), Doxycycline (100%), Erythromycin (100%), Gentamycin (100%) and linezolid (100%) were the most susceptible drugs.

The Antibigram of *Escherichia coli* revealed that the Imipenem (98.41%) was the most susceptible drug followed by Amikacin (90.48%) and Netilmicin (87.30%). The Antibigram of *Klebsiella pneumoniae* revealed that the Imipenem (96.49%) was the most susceptible drug followed by Piperacillin + Tazobactam (64.91%) and Netilmicin (64.91%). The Antibigram of *Proteus* species revealed that the Imipenem (93.75%) was the most susceptible drug followed by Piperacillin + Tazobactam (87.50%) and Netilmicin (87.50%). The Antibigram of *Citrobacter* species revealed that the Imipenem (100.00%) was the most susceptible drug followed by Cefoperazone + sulbactam (93.33%) and Sparfloxacin (86.67%). The Antibigram of *Klebsiella pneumoniae* revealed that the Amikacin (100.00%),

Cefoperazone + Sulbactam (100.00%), Ciprofloxacin (100.00%), Gentamycin (100.00%), Imipenem (100.00%), Netilmicin (100.00%), Piperacillin + Tazobactam (100.00%) and Sparfloxacin (100.00%) were the most susceptible drugs.

The Antibigram of gram-positive cocci revealed that the Vancomycin (100%), teicoplanin (100%) and linezolid (100%) were the most susceptible drugs. Gram-negative Bacilli are susceptible to Imipenem (96.23%), Cefoperazone + Sulbactam (77.22%), Netilmicin (75.47%) and Amikacin (75.00%). All these observation was in agreement with the study by G. Suguneswari et al.^[9]

In our study, the prevalence of ESBLs is 67.45%. A study which was done by Harakuni et al reported a similar high prevalence of the ESBLs (74%).^[10] Laghawe et al reported 19.67% ESBL producers.^[11] It has been proved that the prevalence of the ESBLs varies from country to country and even from institution to institution within the same country. In our study, the AmpC production was seen in 16.98% isolates in concordance with the other studies. It was 17.3% in Kolkata,^[12] 22.9% in a study which was done by Bandekar et al^[13] and a study which was done by Bhattacharjee et al showed 22% AmpC production.^[14] In the present study, the MBL producers were 3.30%. The study which was done by Bandekar et al reported 15.7% MBL producers.^[13] The low prevalence of the MBL producers in our study could be due to the differences in the geographical distribution, which may have produced variations in the prevalence of the β -lactamases which may have been present in the different organisms, which may have given rise to the varied resistance patterns.

The increase in the prevalence of the AmpC, MBL and the ESBL producing isolates may be indicative of the increasing trend of more and more isolates acquiring the resistance, thus rendering the antimicrobial treatment ineffectively.

CONCLUSION

Pyogenic infection has been the major cause of morbidity since long. Emerging multidrug resistant strains is of major concern to treat these conditions. Appropriate and judicious selection of antibiotics by using antibiotic sensitivity data would limit the emerging drug resistant strains in the future to treat these clinical conditions successfully.

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