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Research Article

Bacteriological Profile of Pus and Body Fluid Isolates and Their Antimicrobial Susceptibility Pattern

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Abstract

Introduction: WHO's 2014 report on global surveillance of antimicrobial resistance revealed that antibiotic resistance is no longer a prediction for future; but a pressing concern for the present?

Materials and Methods: A retrospective study was conducted at a tertiary care hospital in Central Delhi. Study involved review of patient's Microbiology laboratory records of bacterial isolates from pus and body fluid specimens from January to December 2012. The data was expressed as percentages and statistically analysed.

Results: Laboratory record of pus and body fluid samples from 5593 patients were analysed. Age of the study group ranged from 1 month to 72 years, with mean age of 25 years. Among the study subjects, 63.6% were males, while 36.4% were females. Staphylococcus aureus was the most frequent isolate (22.3%) followed by Pseudomonas spp. (22.0%), and Klebsiella spp. (18%). Gram positive isolates were most susceptible to levofloxacin, vancomycin, linezolid and teicoplanin. Majority of Gram negative isolates were susceptible to imipenem, meropenem, piperacillin-tazobactam, polymyxin B and colistin. Only 7.3% isolates were susceptible to all the antibiotics tested, while 81.8% isolates were resistant to two or more class of antibiotics.

Conclusion: Without an urgent and coordinated action, the world is heading towards post antibiotic era. Thus it is necessary that studies are conducted to guide the empirical use of antimicrobial agents.

Keywords: Antimicrobial Resistance; Bacteriological Profile; Antimicrobial Agents

Introduction

Pyogenic infections are characterized by local and systemic signs of inflammation, most commonly pus discharge [1]. The commonest pyogenic bacteria are *Staphylococcus aureus, Streptococcus pyogenes, pneumococcus* and *coliform* bacteria such as *Escherichia coli*, *Proteus* species and *Pseudomonas aeruginosa* [2].

The global problem of antimicrobial resistance is particularly pressing in developing countries, where the infectious disease burden is high and cost constraints prevent the wide spread application of newer more expensive agents [3]. Although the evolution of resistant strains is a natural phenomenon, overuse and misuse of antimicrobial agents accelerate the emergence of drug resistant strains.

Patients infected by drug resistant bacteria pathogens suffer a higher morbidity and mortality and thus consume more health-care resources than patients with the same bacteria that are not resistant.

Several similar studies have been conducted in the past. However, due to rising burden of drug resistant bacteria it is imperative that more such studies continue to be performed so that it helps guide clinicians to treat patients with drug resistant organisms well in time.

Materials and Methods

This was a retrospective study conducted at a tertiary care hospital in Central Delhi. A total of 915 consecutive, non-duplicate pus and body fluid samples were analyzed.

Inclusion criteria

Laboratory records of patients with bacterial isolates from pus and body fluid samples tested for antibiotic susceptibility during the period January-December 2012.

Exclusion criteria

1. Laboratory records with incomplete data.
2. Laboratory records with no bacterial growth.
3. Laboratory records with bacterial isolates not tested for antibiotic susceptibility.

Sample processing

In our Microbiology laboratory, all pus and body fluid samples are processed by inoculation on blood agar, Mac Conkey agar and brain heart infusion broth. Inoculated plates and the broth are incubated at 37^0C overnight. Culture plates checked for the bacterial growth next day. All bacterial isolates are examined for colony characteristics, Gram staining, motility and biochemical tests. Biochemical tests employed were oxidase, catalase, nitrate, urea hydrolysis, citrate utilization, sugar fermentation, indole production test and H_2S production on TSI agar.

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Table 1: Distribution of bacterial isolates from pus and body fluid specimens.

Isolates	N (%)
Staphylococcus aureus	1247 (22.3)
Pseudomonas	1230 (22)
Klebsiella spp.	1007 (18)
E.coli	883 (15.8)
Proteus spp.	408 (7.3)
Acnetobacter spp.	205 (3.6)
Providencia	78 (1.4)
Enterococcus	78 (1.4)
Citrobacter spp.	50 (0.9)
Enterobacter spp.	39 (0.7)
Streptococcus pyogens	16 (0.3)
Contaminants	352 (6.3)

Antimicrobial susceptibility testing

In our microbiology laboratory, antibiotic susceptibility testing is performed by modified Stokes disc diffusion method. A suspension of 0.5 McFarland standards is prepared from the colonies of isolated organism and inoculated along with control strains on Mueller Hinton agar plates by sterile swabs. Antibiotic discs are applied on agar and kept for overnight incubation. The antibiotics that were included for testing were cephalexin (30µg), ceftriaxone (30µg), amoxicillin (20µg), ciprofloxacin (5µg), gentamicin (10µg), amikacin (30µg), imipenem (10µg), meropenem (10µg), piperacillin-tazobactam (100µg/10µg), netilmicin (10µg), polymyxin B (300 unit) and colistin (10µg), penicillin (10U), cefazolin (30µg), erythromycin (15µg), clindamycin (2µg), cefoxitin (30µg) vancomycin (30µg), linezolid (30µg) chloramphenicol (30µg), tobramycin (30µg) and aztreonam (30µg) (HIMEDIA Laboratories Pvt. Ltd., Mumbai, India). The discs are used according to standard guidelines and standard (NCTC) strains are used as controls.

The data was expressed as percentages and analyzed by SPSS version 21.p value <0.5 was considered significant.

Results

Laboratory record of pus and body fluid samples from 5593 patients were analyzed. Age of the study group ranged from 1 month to 72 years, with mean age of 25 years. Among these, 63.6% were males, while 36.4% were females. The highest contributor of pus and body fluid specimens was from the Burn and plastic surgery ward (23.1%) followed by Surgery ward (19.4%), Obstetrics and Gynecology ward (17.7%), Medical ward (10.4%), ENT ward (6.4%), Intensive Care Unit (ICU) (7.2%) and Pediatric ward (1.6%). Outpatient department contributed to (14.2%) samples. Out of 5593 specimens received in the Microbiology department, *Staphylococcus aureus* was the most frequent isolate (22.3%) followed by *Pseudomonas* spp. (22.0%), *Klebsiella* spp. (18%), *Escherichia coli* (15.8%), *Proteus* spp. (7.3%), *Acinetobacter* spp. (3.6%), *Providencia* (1.4%) *Enterococcus* spp. (1.4), *Citrobacter* spp. (0.9%), *Enterobacter* spp. (0.7%) and *Streptococcus pyogenes* (0.3%) (Table 1). Gram positive isolates were most susceptible to vancomycin and linezolid. Majority of Gram negative isolates were susceptible to imipenem, meropenem, piperacillin-tazobactam, polymyxin B and colistin. Most resistance of Gram negative isolates was shown to amoxicillin, amino glycoside and cephalosporins (Table 2). In our study, 408 isolates (7.8%) were susceptible to all antibiotics, 248 (4.7%) were resistant to only one antibiotic while 4585 (87.5%) isolates were resistant to two or more antibiotics. Among these 4585 isolates, 3552 (67.8%) were resistant to ≥5 antibiotics tested (Table 3).

Discussion and Conclusion

In our study, age of the study group ranged from 1 month to 72 years, with mean age of 25 years. Among these, 63.6% were males, while 36.4% were females. In another study conducted by Muluye et al, 54.8% study subjects were males while, 45.2% were females [4]. A total of 5593 specimens were received in our study, predominant isolate obtained from pus and body fluid samples was *Staphylococcus aureus* (22.9%) followed by *Pseudomonas* spp. (22.0%), *Klebsiella* spp. (18%), *Escherichia coli* (15.8%), *Proteus* spp. (7.3%), *Acinetobacter* spp. (3.0%), *Providencia* (1.4%) Enterococcus spp. (1.4), Citrobacter spp. (0.9%), Enterobacter spp. (0.7%) and *Streptococcus pyogenes* (0.3%). In another study conducted at a tertiary care hospital in South India, S. aureus was the most frequent isolate (24.29%) followed by P. aeruginosa (21.49%), *E.coli* (14.02%) and Klebsiella spp. (12.15%) among others [5]. In another similar study *S. aureus* (40%) followed by *Klebsiella* spp. (33%), *Pseudomonas* spp. (18%), *E. coli* (16%) and Proteus (7%) were the most frequent isolates [6]. Similar findings were observed among pus isolates from surgical site infection patients in another study [7].

In our study, Gram-positive isolates showed maximum susceptibility to vancomycin and linezolid while Gram-negative isolates were most susceptible to imipenem, meropenem, piperacillin-tazobactam, polymyxin B and colistin. Similar results were obtained in another study conducted by Kaup et al, in 2014 [8]. In a study conducted by Rao et al, Gram positive isolates were found most susceptible to vancomycin, levofloxacin, oxacillin, and clindamycin while Gram negative isolates were most susceptible to piperacillin-tazobactam, levofloxacin, imipenem, aztreonam and amikacin [9]. In our study, S.aureus showed high susceptibility to older drugs like chloramphenicol which means exposure of bacteria to only newly developed antibiotics eliminated resistance against older, out of use antibiotics and thus present bacterial strains have grown susceptible to these out-dated drugs.

In our study, 408 isolates (7.8%) were susceptible to all antibiotics, 248 (4.7%) were resistant to only one antibiotic while 4585 (87.5%) isolates were resistant to two or more antibiotics. Among these 4585 isolates, 3552 (67.8%) were resistant to ≥5 antibiotics tested. In another study conducted by Muluye et al, 11.3% of the isolates were sensitive to all drugs tested, 15.4% were resistance to only one drug, 73.3% were resistant to two or more drugs tested and 20.6% were resistant to more than 5 antibiotics tested [4]. Multidrug resistant isolates have been reported in other studies as well [10,11].

Data from the past several years show an increasing resistance to penicillin, ampicillin and amoxicillin, which were considered 1st line drugs for treatment of pyogenic infection [7]. In most developing countries like India, patients are able to obtain antimicrobials over the counter with or without prescription from a medical practitioner. This could be one of the reasons for high level of resistance among the isolates in the present study.

Table 2: Resistance profile of the bacterial isolates from pus and body fluid samples (% Resistance).

Antibiotics	*S.aureus*	*Pseudomonas aeruginosa*	Enterobacteriaceae	*Acinetobacter* spp.
Penicillin	96.90%	-	-	-
Amoxicillin	-	-	99.10%	88.60%
Piperacillin-Tazobactam	-	18.20%	16.80%	33%
Cefoxitin	44.30%	-	-	-
Cephalexin	41%	-	91.10%	-
Cefazolin	36%	-	-	-
Cefpirome	-	-	93%	-
Ceftriaxone	-	-	87%	79.90%
Ceftazidime	-	68.60%	-	-
Gentamicin	47.70%	73.70%	83.60%	82.70%
Amikacin	30.50%	58%	72.70%	89%
Ciprofloxacin	85.10%	-	-	75.60%
Clindamycin	38.20%	-	-	-
Chloramphenicol	5.40%	-	-	-
Erythromycin	44.80%	-	-	-
Linezolid	0	-	-	-
Vancomycin	0	-	-	-
Aztreonam	-	40%	-	-
Netilmycin	-	72.10%	-	-
Tobramycin	-	70.60%	-	-
Imipenem	-	11.10%	9.40%	15.20%
Meropenem	-	31.80%	31.80%	7.30%
Colistin	-	2.60%	1.60%	1.4
Polymyxin B	-	2.60%	1.60%	1.5

Table 3: Multidrug resistance pattern of bacterial isolates from pus and body fluid samples.

	N (%)					
Organism	R0	R1	R2	R3	R4	≥ P5
Staphylococcus aureus	137 (11.0)	180 (14.4)	259 (20.8)	170 (14)	201 (16.1)	300 (24.1)
Pseudomonas spp.	228 (18.5)	43 (3.5)	27 (2.2)	38 (3.1)	206 (16.7)	688 (55.9)
Klebsiella	27 (2.7)	10 (1)	27 (2.7)	0	27 (2.7)	916 (90.9)
E. coli	0	15 (1.7)	7 (0.8)	0	0	861 (97.5)
Proteus	0	0	17 (4.2)	0	54(13.2)	337 (82.6)
Acinetobacter	0	0	0	0	0	205 (100)
Providencia	0	0	0	0	0	78 (100)
Enterococcus	0	0	0	0	0	78 (100)
Citrobacter	0	0	0	0	0	50 (100)
Enterobacter	0	0	0	0	0	39 (100)
Streptococcus	16 (100)	0	0	0	0	0

R0-Suscceptible to all antibiotics tested, R1, R2, R3, R4, R5- Resistant to one, two, three, four and five or more antibiotics.

Multidrug resistance may be due to empirical use of broad-spectrum antibiotics, non-adherence to hospital antibiotic policy, chronic course of wound and frequent hospital admission. A longer duration of illness and treatment increases healthcare costs as well as predisposes patients to more serious hospital acquired infection.

Knowledge of common pathogens and their resistance status can guide clinician to choose appropriate antibiotics for empirical treatment of patients. Limitation of this study was that limited number of antibiotics was used to test some isolates. Also, since it is a retrospective study, some data registered was incomplete and therefore not included.

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