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Original Research Article

Microbial Profile and Antibiotic Resistance Pattern of The Bacterial Isolates

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Abstract:

Objectives: The present study was to evaluate the microbial profile and antibiotic resistance pattern of the bacterial isolates at tertiary care centre.

Methods: All the bacterial isolates of interest were subjected to antibiotic susceptibility testing to different antibacterial agents by standard Kirby-Bauer disc diffusion method using commercially available discs (Hi-media, Mumbai, India) as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The quality control of the antibiotic discs was done by using ATCC strains of E Coli 25922 and S. aureus 25923. The MDR categorizations were done.

Results: The most frequently encountered isolates from all samples were E. coli (45.71%), Streptococcus spp. (20%), Staphylococcus aureus (17.14%), Citrobacter spp. (2.86%), Klebsiella spp. (2.86%), Pseudomonas aeruginosa (5.71%), Moraxella catarrhalis (2.86%) and Micrococcus spp. (2.86%). MDR accounted for 82.86% of the total bacterial isolates, higher among the Gram negatives (37.93%) compared to Gram positives (62.07%). Mono drug resistant was seen in 9.52% gram negative and gram-positive bacteria.

Conclusions: Multidrug resistance is greater in bacterial isolates. Most isolates are Escherichia coli. Multi drug resistance is more common in gram negative bacteria. Hence, drug resistances are higher due to misuse of antibiotics, over-prescription, self-medication, and over-the-counter sales, the absence of standardized guidelines for antibiotic usage and poor sanitation and hygiene. So that, we should organise health check-up camp time to time in community for awareness of the misuse of antibiotics and drug resistance.

Keywords: Bacterial isolates, Miss use of antibiotics, Multi Drug Resistance.

Introduction

Antimicrobial resistance (AMR) is a global health and development threat that emerged as one of the major public health problems of the 21st century and warns against the effective prevention and treatment of an ever-increasing range of infections [1]. It has been noted over many decades that bacteria causing common or severe infections have developed resistance to every new antibiotic entering the market [2, 1].

The World Health Organisation (WHO) has recognized for a long time that a better, more coordinated global effort is required to combat AMR. In 2001, the WHO Global Strategy for Containment of Antimicrobial Resistance provided an agenda of interferences to slow the emergence and reduce the distribution of antimicrobial-resistant microorganisms [3]. In 2012, WHO published 'The Evolving Threat of Antimicrobial Resistance: Options for Action [4], proposing a combination of interventions that include strengthening health systems and surveillance, improving the use of antimicrobials in hospitals and the community, infection prevention and control, encouraging the development of appropriate new drugs and vaccines, and political commitment [4].

WHO has published a global list of antibiotic-resistant bacteria [5] to prioritize research and development of new antibiotic treatments. Among those top human pathogens, methicillin-resistant Staphylococcus aureus (MRSA), now endemic in the community, presents critical therapeutic difficulties due to its high adaptability [6, 7]. In Latin America, 45% of the S. aureus isolates from nine countries collected in 2011 – 2014 showed methicillin resistance, but with important regional variations. Those same isolates exhibited high rates of resistance to other commonly-used antibiotics [8].

Other important pathogens on the WHO list are the Enterobacteriaceae family and Pseudomonas aeruginosa. In a study published in 2017, members of the Enterobacteriaceae family showed a high prevalence of production of extended spectrum b-lactamase (ESBL) enzymes in Latin America and

the Caribbean, especially Escherichia coli and Klebsiella spp. [9]. Additionally, 26% of the P. aeruginosa isolates exhibited a multidrug-resistant (MDR) phenotype [9]. A short report published recently analyzed resistance patterns of microorganisms isolated from pediatric patients in DR [10]. It reported that 50% of the gram-negative bacteria were resistant to at least one third-generation cephalosporin; 17% to one or more carbapenem drugs; and for S. aureus isolates, 58% were resistant to methicillin [10]. Objectives of our study was to evaluate the microbial profile and antibiotic resistance pattern of the bacterial isolates at tertiary care centre.

Material & Methods

The present study was conducted in the Department of Microbiology, Lord Budha Medical Collegel, Saharsha, Bihar during a period from January 2025 to May 2025.

Study Design and Collection of Specimen: A total of 145 clinical specimens including post operative swab, throat swab, vaginal swab, sputum, pus, stool, urine and wound swab samples were processed.

Isolation and Identification of Microorganisms: For isolation of desired pathogens, all the specimens were inoculated on the MacConkey agar, blood agar, chocolate agar, and mannitol salt agar media plates, followed by overnight incubation at 370C. Chocolate agar plates were incubated in CO2. Identification of the bacteria of interest was performed by series of biochemical tests following standard methods including the triple sugar iron agar (TSI) test, indole motility test, citrate utilization test, catalase test, oxidase test, coagulase test etc [11].

Assay of Bacterial Antibiotic Susceptibility Pattern: All the bacterial isolates of interest were subjected to antibiotic susceptibility testing to different antibacterial agents by standard Kirby-Bauer disc diffusion method [12] using commercially available discs (Hi-media, Mumbai, India) as per Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. The quality control of the antibiotic discs was done by using ATCC strains of E Coli 25922 and S. aureus 25923. The MDR categorizations were done based on the interim standard proposed by Magiorakos et al. [14]. The antibiotics tested were as classified in table 1.

Table 1: Showing the classification of antimicrobial agents.

Name of the Group	Name of the antimicrobials
Cephalosporins	Cefuroxime (30 µg), Cefotaxime (30µg), Cepfodoxime (10µg), Ceftriaxone (30µg), Cefixime (5 µg), Cephalexin (30 µg), Ceftriaxone/sulbactum (45µg)
Aminoglycosides	Amikacin(30µg), Gentamicin(10µg).
Fluoroquinolones	Ciprofloxacin(5µg), Levofloxacin(5µg), Prulifloxacin(5µg), Ofloxacin(5µg)
Penicillins	Amoxycillin/clavulunate(30 µg), Amoxycillin (10µg), Ampicillin/sulbactum(20µg), Ampicillin(10µg), Piperacillin/tazobactum(110µg), Oxacillin (1µg)
Carbapenems	Imipenem(10µg)
Macrolides	Azithromycin(15µg), Erythromycin(15µg)
Lincosamides	Clindamycin(2µg)
Nitrofurans	Nitrofurantoin(300µg)
Sulphonamides	Cotrimoxazole(25µg)

Statistical Analysis: Data was analysed using MS-Office software. All data was tabulated and percentages were calculated.

Results

A total of 145 samples were received of which urine specimens (n=123) accounted for the highest in number followed by sputum (n=3), throat swab (n=4), pus (n=1), wound swab (n=1), post operative wound swab (n=1), vaginal swab (n=2).

Microbial Growth Status of the Samples: Of total 145 samples 90 samples showed no growth, 12 insignificant growths, 3 mixed growths, 5 normal floras and 1 sample gave Candida spp. Only 35 samples gave significant bacterial growth.

Table 2: Sample Wise Distribution of Bacterial Isolates

Sample type	Klebsiella spp.	E. coli	Citrobacter spp.	P. aeruginosa	M. catarrhalis	Micrococcus spp.	Strept. spp.	S.aureus	Total
Sputum	0	0	0	0	1(33.33%)	0	2(66.67%)	0	3
Urine	1(4.17%)	16(66.66%)	1(4.17%)	2(8.34%)	0	1(4.17%)	1(4.17%)	2(8.34%)	24
Throat swab	0	0	0	0	0	0	4(100)	0	4
Pus	0	0	0	0	0	0	0	1(100%)	1
Vaginal swab	0	0	0	0	0	0	0	2(100%)	2
Wound swab	0	0	0	0	0	0	0	1(100%)	1
Total	1(2.85%)	16(45.71%)	1(2.85%)	2(5.71%)	1(2.85%)	1(2.85%)	7(20%)	6(17.14%)	35

Microbial profile of pathogens: The most frequently encountered isolates from all samples were E. coli (45.71%), Streptococcus spp. (20%), Staphylococcus aureus (17.14%), Citrobacter spp. (2.86%), Klebsiella spp. (2.86%), Pseudomonas aeruginosa (5.71%), Moraxella catarrhalis (2.86%) and Micrococcus spp. (2.86%). All the isolates from throat swab and sputum samples were Streptococcus spp. except one Moraxella catarrhalis in sputum. Vaginal swab, wound swab and pus showed the growth of Staphylococcus aureus as the only predominant pathogen isolate.

Table 3: Drug resistant bacterial isolates

Organism	Multi-drug resistant (MDR)	Mono-drug resistant (MR)	Non- Resistant (NR)	Total
Klebsiella spp.	1(100%)	-	-	1(2.86%)
Escherichia coli	13(81.25%)	2(12.5%)	1(6.25%)	16(45.71%)
Citrobacter spp.	1(100%)	-	-	1(2.86%)
Pseudomonas aeruginosa	2(100%)	-	-	2(5.71%)
Moraxella catarrhalis	1(100%)	-	-	1(2.86%)
Staphylococcus aureus	3(50%)	2(33.33%)	1(16.67%)	6(17.14%)
Micrococcus spp.	1(100%)	-	-	1(2.86%)
Streptococcus spp.	7(100%)	-	-	7(20%)
Total	29(82.86%)	4(11.43%)	2(5.71%)	35(100%)

Antibiotic Resistance Patterns Among the Bacterial isolates: MDR accounted for 82.86% of the total bacterial isolates, higher among the Gram negatives (37.93%) compared to Gram positives (62.07%). Mono drug resistant was seen in 9.52% gram negative and gram-positive bacteria. Nonresistant to drug was seen in only 4.76% gram-negative and gram-positive bacteria.

Table 4: Resistance category and the bacterial groups

Resistance Category	Bacterial Group		Total
	Gram Negative	Gram Positive	
Multi-drug resistant (MDR)	18(85.71%)	11(78.57%)	29(82.86%)
Mono-drug resistant (MR)	2(9.52%)	2(14.23%)	4(11.43%)
Non- Resistant (NR)	1(4.76%)	1(7.14%)	2(5.71%)
Total	21(60%)	14(40%)	35(100%)

Discussions

Serious infection caused mostly by gram-negative pathogens causes significant morbidity, and emerging multidrug resistance patterns increase morbidity and mortality even further, especially in critically ill patients [15,16]. According to the Centers for Disease Control and Prevention, antimicrobial resistance kills over 700,000 people worldwide each year, with that figure expected to rise to 10 million by 2050 [17]. Physicians are increasingly challenged to provide their patients

with effective antibiotic regimens that do not result in further drug resistance. Antibiotic resistance is on the rise, and as a consequence, serious public health issues are arising [18, 19].

In the present study, significant bacterial growth was observed in 24.14% of the total samples received, near about similar to a recent study [20]. Resistant to at least one of the commonly used antibiotics was detected in 90.7% of the isolates which is very high figure compared to 70% as shown by a similar study in Bangladesh [21]. The overall prevalence of MDR was 83.7% against 62.8% shown by earlier report [20]. This may be due to small sample size or difference in the kind of study site.

In the present study, out of 21 gram-negative organism, MDR prevalence among enterobacteriaceae was 85.71%, nearly similar to the studies in Ethiopia (85.5%,87.4%) [22,23] while higher than the studies in Nepal (40.1%, 64.04%) [24,25]. This variation in the prevalence rate could be due to increased incidence of MDR strains with time, difference in study period and study population. All the E. coli isolates were from urine sample constituting the majority (45.71%) among the uropathogens slightly more compared to similar study from North India (56.8%).[26]

In the present study, 81.25% E. coli were MDR which is similar to a study from South India (82.6%) [27].

Among 21 enterobacteriaceae isolates 1 (4.76%) was found to be carbapenem resistant, similar to other studies in India (5.4%) [28] and Bangladesh (4.8%) [29]. In the meantime, another study in India is seen to have reported carbapenem resistant prevalence rate of up to 12.9% [30]. This difference in the prevalence of carbapenem resistance enterobacteriaceae in various studies may be due to increased trends in the utilization of carbapenems and other broad-spectrum antibiotics, traditional practices, transfer of patients from the place of high incidence to another. All the two isolates of Pseudomonas aeruginosa were found to be MDR and all isolates of Citrobacter spp. One isolates of Klebsiella spp. and Moraxella catarrhalis were also found to be MDR. These later findings are not in concordance with the other studies [20,31-32] as because of small study sample, targeted patient group basically having psychiatric complaints and study period.

The incidence of Gram-positive organism was reported to be 14(40%) which is consistent with other studies that have shown the higher incidence of Gram-negative organism compared to that of Gram positive [33-34].

Out of 14 Gram positive organism S. aureus accounted for 42.86%, Streptococcus spp. 50% and Micrococcus spp. 7.14%, of which 78.57% of gram-positive isolates were MDR, very huge difference in figures among the isolates compared to previous findings [35,36]. This variation may be due to the small sample population as well as due to limited number of visits with related complaints as the study was carried out in a psychiatric setting.

The overuse of antibiotics can be due to a combination of factors, including physiological changes leading to recurrent infections and frequent exposure to multidrug resistance bacteria in long-term care facilities [37,38]. The higher number of comorbidities among older patients causes more hospitalizations, a setting where they get exposed to multidrug-resistant bacteria [39, 40].

Antimicrobial resistance (AMR) is a phenomenon that occurs when different groups of microorganisms shift over a period and no longer respond to medicines that have been effective so far [41]. Although AMR is a natural process, the public health emergency due to the uncontrolled spread of this phenomenon is the consequence of overuse and/or misuse of antibiotics [42]. However, other driving factors are also mainly responsible for the increase in its prevalence. These include poor community hygiene, poor infection control in hospitals and clinics, the accumulation of antibiotics in the environment, and their use in the animal and food industries [2, 43].

Conclusions

The present study concluded that the multidrug resistance is greater in bacterial isolates. Most isolates are Escherichia coli. Multi drug resistance is more common in gram negative bacteria. Hence, drug resistances are higher due to misuse of antibiotics, over-prescription, self-medication, and over-the-counter sales, the absence of standardized guidelines for antibiotic usage and poor sanitation and hygiene. So that, we should organise health check-up camp time to time in community for awareness of the misuse of antibiotics and drug resistance.

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