Status of Multidrug Resistant Bacterial Strains Isolated from Urinary and Respiratory Specimens at Tribhuvan University Teaching Hospital

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Abstract

Introduction The progressive increase in antibiotic resistance among pathogens in developing countries is becoming a critical area of concern globally. Microbial resistance to drugs of different classes has arisen in a multitude of species which may complicate the therapeutic management of infections.

Objectives To assess the breadth of multidrug resistant bacterial pathogens isolated from urine and sputum samples.

Methods In this cross-sectional study, the clinical samples were subjected to routine processing following standard protocol. The antimicrobial susceptibility of bacterial isolates was determined by National Committee for Clinical Laboratory Standards (NCCLS) recommended by Kirby-Bauer method.

Results All together, 325 samples were examined, of which 218 were urine samples and 107 were sputum samples. Of total urine samples, 60 (27.5%) showed significant growth and 68.3 percent (41/60) were MDR-strains. *Escherichia coli* (N=37) was found to be the most predominant isolate from urine sample, 64.8 percent of which were MDR-strains. Similarly, out of total processed sputum samples, 45 (43.3%) showed significant growth, out of which, 48.9 percent (22/45) were MDR-strains. *Haemophilus influenzae* (N=13) was the most predominant isolate, followed by *Klebsiella pneumoniae* (N=10). No isolate of *H. influenzae* was found to be MDR-strain, while 70 percent of *K. pneumoniae* were MDR-strains. Ceftazidime and Amikacin were found to be the most effective drugs for gram negative MDR isolates; while Vancomycin and Cloxacillin were the drugs of choice for gram positive MDR isolates from urine and sputum samples respectively. Significant association was found between multidrug resistance and hospitalization of patients ($P<0.05$), whereas no association was observed between multidrug resistance and gender ($P>0.05$).

Conclusion The findings signify the key issue of growing trend of drug resistance among pathogenic bacteria in clinical samples and suggested that stringent guidelines should be established for antibiotic policy within hospitals, encouraging prudent use of antimicrobials.

Key Words Multidrug resistance, ß-lactamase, NCCLS recommended Kirby-Bauer method.

Introduction

The selective pressure resulting from the extensive use of antibiotics over the last 50 years has led to the emergence of bacterial resistance, and to the dissemination of resistance genes among pathogenic microorganisms. The progressive emergence and rapid dissemination of antibiotic resistance and its association with the use and consumption of antibiotics constituted a major health concern and have been considered a global crisis[1]. The prevalence of antimicrobial agent-resistant bacteria has dramatically increased in hospitals worldwide during the last few years[2].

Acquired antimicrobial resistance is a growing worldwide problem due to the increasing use of antimicrobials in humans, animals, and agriculture. More so in developing countries like ours, the situation is particularly serious for reasons such as availability of antimicrobials outside of recognized

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treatment centers, lack of guidelines regarding the selection of drugs, correct prescribing, and information about drug resistance, and overcrowding and poor hygiene[1]. Several mechanisms are responsible for the ability of microorganisms to tolerate antibiotics, and the incidence of resistance to these compounds within bacterial species has increased since the commercial use of antibiotics became widespread[2].

Therefore the continuous and ongoing nature of resistance emergence and dissemination dictates that reliable laboratory procedures be used to detect resistance as an aid to managing the infected patient and as a means for monitoring the changing resistance trends among clinically relevant bacteria.

Methods

The study site for this study was the Bacteriology Laboratory, Tribhuvan University Teaching Hospital, Maharajgunj, Kathmandu. The study was carried out from March to May 2005. All together, 325 samples were received in the laboratory, of which 218 were mid-stream urine samples and 107 were sputum samples. The history of patients was noted according to a questionnaire. The samples received in the laboratory were processed following standard laboratory techniques[3].

Sample Processing

Semi quantitative culture by standard loop method was followed for urine samples by inoculating onto 5 percent blood agar (Oxoid, England) and MacConkey agar (Oxoid, England). The plates were incubated aerobically at 35-37^{0}C overnight. The significant bacterial isolates (>10^5 CFU/ml) were identified following standard microbiological techniques.

Sputum samples were accepted for further processing according to the American Society for Microbiology (ASM) criteria[4]. The accepted samples were inoculated in the MacConkey agar, 5 percent blood agar and chocolate agar plates. In the chocolate agar plate, a 5 µg Optochin disc and a 10 U Bacitracin disc were added to screen out *Streptococcus pneumoniae* and *H. influenzae* respectively, and the plates were incubated at 37^{0}C for overnight in candle jar; whereas, the MacConkey and blood agar plates were incubated under aerobic condition. The significant isolates were identified following standard microbiological techniques.

The antimicrobial susceptibility of bacterial isolates was determined by National Committee for Clinical Laboratory Standards (NCCLS) recommended Kirby-Bauer method[5]. The antibiotic discs and culture media used were from Oxoid Limited, Basingstoke, Hampshire, England.

Results

Out of 218 urine samples, 60 (27.5%) showed significant growth, and among the 60 isolates, 41 (68.3%) were multi-drug resistant. Similarly, out of 107 sputum samples received, only 104 (97.2%) samples met the American Society for Microbiology criteria, so only these were considered for further processing, and out of these, 43.3 percent (45/104) showed significant growth, out of which, 48.9 percent (22/45) were multi-drug resistant. The results are shown in table 1.

Table 1: Pattern of clinical samples and status of MDR strains

Specimen	No. of samples	Significant growth No. (%)	MDR strains No. (%)
Urine	218	60 (27.5)	41 (68.3)
Sputum	104	45 (43.3)	22 (48.9)
Total	322	105 (32.6)	63 (60.0)

Among the 60 urinary isolates, Gram negative bacteria constituted 50 (83.3%) of the total isolates, among which 33 (66%) were MDR. Among Gram negatives, *E. coli* was the most frequently isolated species with 37 (61.7%) isolates, among these, 24 (64.9%) were found to be MDR-strains. Gram positive organisms constituted 10 (16.7%) of total isolates, and 8 (80%) of them were MDR. *Staphylococcus aureus* constituted 6 isolates, 4 (66.7%) of these were MDR; whereas all 4 *Enterococcus faecalis* isolates were found to be MDR-strains. The results are shown in table 2.

Table 2: Pattern of microbial isolates from urine sample

Organisms	MDR (%)
Gram Positive Bacteria	
(N=10)	**(N=8)**
S. aureus (N=6)	4 (66.7)
E. faecalis (N=4)	4 (100.0)
Gram Negative Bacteria	
(N=50)	**(N=33)**
E. coli (N=37)	24 (64.9)
K. pneumoniae (N=4)	3 (75.0)
Klebsiella oxytoca (N=3)	1 (33.3)
P. aeruginosa (N=2)	2 (100.0)
Proteus mirabilis (N=2)	1 (50.0)
Citrobacter freundii (N=2)	2 (100.0)

Among the common antibiotics used against MDR strains from urine sample, ceftazidime was the most effective with a susceptibility of 66.67 percent for Gram negative isolates; whereas, Vancomycin (used only for *E. faecalis*) was 100 percent effective against this isolate. The results are shown in table 3.

Table 3: Antibiotic susceptibility pattern of MDR strains from urine sample

Gram negative bacteria

Antibiotic used	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ampicillin	1	3.2	0	0.0	30	96.8	31
Ciprofloxacin	9	27.3	0	0.0	24	72.7	33
Cephalexin	5	15.1	5	15.1	23	69.7	33
Norfloxacin	10	30.3	1	3.0	22	66.7	33
Nitrofurantoin	21	63.6	4	12.1	8	24.2	33
Ceftazidime	22	66.7	0	0.0	11	33.3	33
Amikacin	1	50.0	0	0.0	1	50.0	2

Gram positive bacteria

Antibiotic used	Sensitive		Intermediate		Resistant		Total
	No.	%	No.	%	No.	%	
Ampicillin	3	37.5	0	0.0	5	62.5	8
Ciprofloxacin	2	25.0	1	12.5	5	62.5	8
Cephalexin	2	25.0	0	0.0	6	75.0	8
Oxacillin	1	12.5	0	0.0	7	87.5	8
Erythromycin	2	25.0	0	0.0	6	75.0	8
Vancomycin	4	100.0	0	0.0	0	0.0	4

Among the 45 isolates from sputum sample, Gram negative bacteria were predominant constituting 34 (75.6%) of the total isolates, among these 47.1 percent (16/34) were MDR. *H. influenzae* was the most frequently isolated species with 13 isolates, however, among these, none were found to be a MDR-strain. Similarly, out of 10 isolates of *K. pneumoniae,* 7 (70%) were MDR. Gram positive organisms constituted 11 (24.4%) of total isolates, and 6 (54.5%) of them were MDR. *S. aureus* constituted 6 isolates, 5 (83.3%) were MDR. The results are shown in table 4.

Table 4: Pattern of microbial isolates from sputum sample

Organisms	MDR (%)
Gram Positive Bacteria	
(N=11)	
S. aureus (N=6)	5 (83.3)
S. pneumoniae (N=5)	1 (20.0)
Gram Negative Bacteria	
(N=34)	
H. influenzae (N=13)	0 (0.0)
K. pneumoniae (N=10)	7 (70.0)
P. aeruginosa (N=7)	5 (71.4)
Others (N=4)	4 (100.0)

OTHERS: *Escherichia coli* (N=2), *Citrobacter freundii* (N=1), *Acinetobacter calcoaceticus* (N=1)

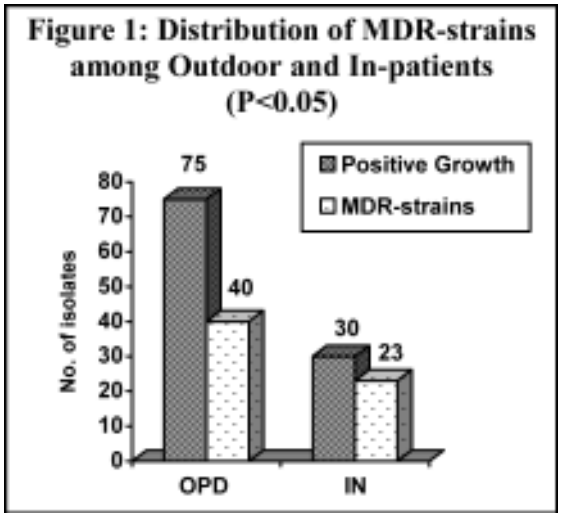
Among the common antibiotics used against all MDR strains from sputum sample, amikacin was found to be most effective for Gram negative isolates with a susceptibility of 81.2%, whereas, Cloxacillin was 100% effective against the Gram positive isolates. The results are shown in table 5.

Table 5: Antibiotic susceptibility pattern of MDR strains from sputum sample

Gram negative bacteria							
Antibiotic used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Ampicillin	0	0.0	0	0.0	11	100.0	11
Ciprofloxacin	3	18.7	0	0.0	13	81.2	16
Cephalexin	0	0.0	0	0.0	16	100.0	16
Gentamicin	6	37.5	0	0.0	10	62.5	16
Ceftriaxone	0	0.0	0	0.0	5	100.0	5
Ceftazidime	12	75.0	1	6.2	3	18.7	16
Amikacin	13	81.2	0	0.0	3	18.7	16
Gram positive bacteria							
Antibiotic used	**Sensitive**		**Intermediate**		**Resistant**		**Total**
	No.	**%**	**No.**	**%**	**No.**	**%**	
Ampicillin	1	16.7	0	0.0	5	83.3	6
Ciprofloxacin	1	16.7	0	0.0	5	83.3	6
Cephalexin	1	16.7	0	0.0	5	83.3	6
Cloxacillin	3	50.0	0	0.0	3	50.0	6
Erythromycin	2	33.3	0	0.0	4	66.7	6

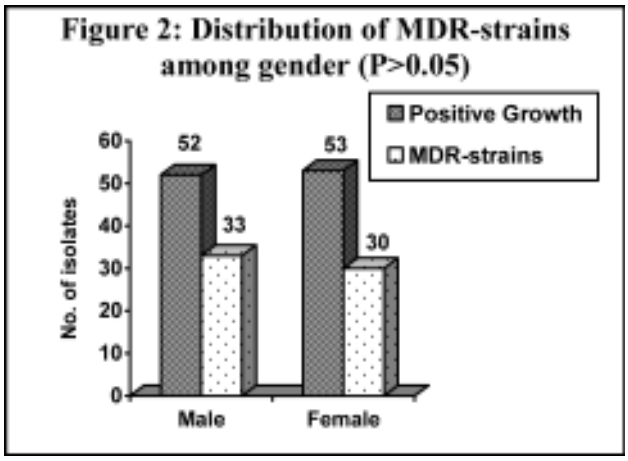
A higher proportion of MDR-strains was seen among the in-patients, which was found to be statistically significant (Figure 1).

Figure 1: Distribution of MDR-strains among Outdoor and In-patients (P<0.05)



A slightly higher proportion of MDR-strains was seen among the male patients, however this was not found to be statistically significant (Figure 2).

Figure 2: Distribution of MDR-strains among gender (P>0.05)



Discussion

The defining criterion for an isolate to be a multidrug resistant strain in this study was set as resistance to two or more drugs of different structural classes[6]; 68.3 percent of total isolates from urine and 48.9 percent of total isolates from sputum were thus found to be MDR strains in the present study.

In this study, among the total *E. coli* isolates from urine, 64.9 percent were found to be MDR-strains. Other studies have demonstrated similar findings[7, 8]. Similarly, 75 percent of *K. pneumoniae* isolates from urine and 70 percent of the same from sputum in this study were found to be MDR strains. The classical TEM-1, TEM-2, and SHV-1 ß-lactamases predominantly mediate resistance to ß-lactam antimicrobial agents among Gram-negative rods[9]. Whereas, mutations at the target site (*gyrA and parC* genes) appear to be the major mechanism for fluoroquinolone resistance in *E. coli*[10]. Numerous case-control studies have established previous fluoroquinolone exposure as a significant risk factor for the isolation of fluoroquinolone-resistant *Enterobacteriaceae* from clinical specimens[11, 12].

Among the Gram-positive isolates in this study, 83.3 percent of the *S. aureus* isolates and all 4 isolates of *E. faecalis* from urine were found to be MDR-strains. Among the two different mechanisms of resistance of *S. aureus* to ß-lactam antibiotics: the first is the production of penicillinase. The second mechanism,

methicillin resistance, is associated with the production of an additional penicillin-binding protein (PBP 2' or 2a) with low affinity for ß-lactam antibiotics[13]. Although, Vancomycin was found to be 100 percent effective in this study, the prevalence of VRE has been increasing in the United States in the past 15 years[14]. Notably, in 2002, a clinical isolate of Vancomycin-resistant *S. aureus* was isolated in Michigan[15].

No isolate of *H. influenzae*, the most predominant isolate from sputum, was found to be MDR in our study. However, the first description of ß-lactamase-mediated ampicillin resistance in *H. influenzae* was reported in 1974[16], and in 1980, the problem of antibiotic resistance among *H. influenzae* isolates was complicated further by the description of the ß-lactamase negative but ampicillin intermediate or resistant strains[17].

In this study, both the isolates of *P. aeruginosa* from urine and 71.4 percent of the same from sputum sample were found to be MDR-strains. Infection with *P. aeruginosa* is a serious problem affecting hospitalized patients, particularly those who are critically ill and immunocompromised, such as patients with cystic fibrosis[18].

In the present study, 20 percent of *S. pneumoniae* isolates were found to be MDR**.** The first isolation of penicillin-resistant pneumococci was reported in Australia[19]. The resistance pattern displayed by *S. pneumoniae* isolates were reported in various other studies[20, 21].

Significant association was found between multidrug resistance and hospitalization of patients ($P<0.05$). This may be due to the fact that the hospital-strains of pathogens are subjected to selective pressure resulting from the extensive use of antibiotics to a much larger extent than the community strains. This result is in agreement with a previous study[7]. The health care system has been greatly impacted by the emergence of antibiotic-resistant Gram-negative infections and the literature is replete with evidence that the presence of antibiotic-resistant *Enterobacteriaceae* is associated with longer hospital stays, greater use of additional antibiotics, and higher mortality[22].

The emergence of resistant pathogens is an important factor in the morbidity and mortality of hospitalized patients. In the face of this growing resistance among these organisms, the selection of the correct antimicrobial interventions, based on correct identification and susceptibility test data, has become increasingly challenging[23].

Overall, the findings of this study signify the key issue of growing trend of drug resistance among pathogenic bacteria in clinical samples and suggest that stringent guidelines should be established for antibiotic policy within hospitals; so that unnecessary use of antibiotics could be minimized, and that the clinical use of antibiotics should be improved by addressing over prescription, incorrect prescription, and non-compliance by patients; and discouraging non-clinical use of antibiotics such as in animal feed. Genotypic characterization of the MDR strains should be carried out as an extension to this type of study in order to establish the location of drug resistance genes and to characterize the mechanism of drug resistance.

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