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Multidrug Resistance Bacteria in Different Clinical Samples in National Medical College and Teaching Hospital Birgunj, Nepal

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ABSTRACT

A cross-sectional study was conducted in National Medical College and Teaching Hospital, Birgunj to study of multidrug resistant (MDR) bacteria in different clinical samples. Altogether 281 clinical samples were investigated in this study where 227 were urine samples and 54 were pus samples during the working period from March to September, 2010. A total of 76 (33.48%) urine samples and 37 (68.52%) pus samples were found to be positive. Analysis of the sample showed that UTI (Urinary tract infection) was more common in female as compared to male. It was found that 19(65%) E. coli were multidrug resistant out of 49 isolates isolated from 281 samples (including Urine, Pus), 9 (54.50%) Staphylococcus aureus were multidrug resistant out of 34 isolates. Similarly 5 (94.44%) Klebsiella pneumonia out of 11 isolates, 2 (58.33%) Pseudomonas aeruginosa out of 7 isolates, 1(50%) Enterococcus faecalis out of 2 isolates were multidrug resistant. Antimicrobial drug resistance is a major problem in Nepal. This study shows that a good percentage of people were infested by multi-drug resistant bacterial agents. The information provided in the study may be useful in improving control programmes directed against infectious disease in the Terai region of Nepal.

Keywords: Antimicrobial drug, Clinical samples, Gram positive, Gram negative, Multidrug resistance

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INTRODUCTION

The progressive emergence and rapid dissemination of antimicrobial resistance is one of the biggest challenges facing global public health [1]. Failure to adhere, to proper infection control technique, unrational use of antibiotics, unhygienic practices, increased uses of antibiotics in animal and plants and more so availability of antibiotics without prescription and counterfeit products of dubious quality in developing countries have resulted in spread of antimicrobial resistance[1,2] and selection of multidrug resistant bacterial pathogens [3]. The broad use of antibiotics had created a strong selective pressure, which consistently had resulted in the survival and spread of resistance that has evolved with the increased number, volume and diversity of antimicrobial applications [5].

Substantial cause substantial resistance and which requires a multidisciplinary approach and closer collaboration among health care members in hospitals, pharmacist, infection control practitioners and infectious disease specialists. They can reduce the treatment failures and minimize the spread of multidrug-resistance organisms between the hospital environment and the community. The emergence of antimicrobial resistance pathogens now treats the discovery of potent antimicrobial agents. Antimicrobial resistance has resulted in increased morbidity and mortality as well as health care costs. Yearly expenditures arising from drug resistance in the United States are $4 billion and are rising [4].

In Nepal, the resistant pathogens are more common because the misuse of antibiotics and people fail to finish the full course of treatment. Patient then stockpile the leftover doses and medicate themselves or their family in less than therapeutic amounts. In both circumstances, the improper dosing will fail to eliminate the disease agent completely and will furthermore; encourage growth of most resistant strains. The important factors associated with resistant bacteria are poor resources for infection control and lack of personnel trained in controlling infection in hospital.

MATERIALS AND METHODOLOGY

This is a prospective study carried out in the Microbiology department of National Medical College and Teaching Hospital, (NMC&TH) Birgunj between March 2010 to September 2010.

Specimen size and specimen types:

A total of 281 different samples including Urine (227) and Pus (57) sent for routine culture and antibiotic susceptibility tests were processed during the study period.

Culture of the specimen:

Urine specimens were cultured by semi quantitative culture technique. A loopful of well mixed uncentrifused urine sample was inoculated onto blood agar (BA) and MacConkey agar (MA) using sterile calibrated loop. The plates were incubated in ambient atmosphere at 37°C for 24 to 48 hours.

Pus sample was aseptically inoculated on to blood agar (BA), MacConkey agar (MA) andMannitol salt agar (MSA). The BA plate was incubated at 5-10% CO_2 rich atmosphere whereas MA and MSA in ambient atmosphere at 37°C for 24 to 48 hours.

Identification and antibiotic susceptibility test:

Identification of significant isolates was done by standard microbiological techniques.

Antibiotic susceptibility testing:

The isolates were then subjected to antibiotic sensitivity testing by the disc diffusion method on Mueller-Hinton agar according to the National Committee for Clinical Laboratory Standards and Manual of Antimicrobial Susceptibility Testing guidelines [11.12.19]. Commercially available antimicrobial discs were used in the study and included in the table 3. Plates were incubated at 35-37°C. Zones of inhibition were interpreted as resistant or sensitive using the interpretative chart of the zone sizes of the Kirby – Bauer sensitivity test method as described by Cheesbrough. Interpretation of results was done using the zone of inhibition sizes. Zones of inhibition of _ 18 mm were considered sensitive, 13-17 mm intermediate and < 13 mm resistant [11,12,19, 21]

Criterion for multi drug resistant:

In the present study the defining criterion for an isolate to be Multidrug resistant (MDR) was set as resistant to two or more drugs of different structural classes.

RESULTS

The microbiological characteristics of the different organisms used in this study are presented in Table 1. This shows the cultural, morphological and biochemical characteristics of these isolates. The isolates were confirmed to be, Escherichia coli, Klebsiella pneumoniae, K. oxytoca, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter species , Staphylococcus aureus , S. saprophyticus, and Enterococcus faecalis. Table 2 shows the microbial pattern of multi drug resistance isolates. Out of total 66 (64.0) isolates in urine 26 (39.3%) isolates were found to be multidrug resistance .Likewise out of total 37(35.9) isolates from pus sample 10 (27.0) isolates were found to be multidrug resistant.

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Table: 1 Morphological characterization for bacterial isolates:

Parameters	Isolates								
	EC	KP	KO	PM	PA	CI	SA	SS	EF
Gram reaction	_	_	_	_	_	_	+	+	+
Cellular morphology	Straight rod	Rod	Rod	Small rods	Small rods	Straight rods	Cocci	Cocci	Cocci
Growth on Blood agar (colony)	Large,flat spreading & circular mucoid	Large Greyish-white mucoid	Large Greyish-white mucoid	Swarming with fishy smell	Green ish	Smooth moist translucent or opaque	Creamy white	Pale orange pigment	Small convex
Growth on MacConkey agar	Smooth Red/Pink	Pink mucoid	Pink mucoid	Pale	Pale	Pale/Pink (late lactose fermenter)	Small pink	Small pink	Pin point magenta
Growth on Manitol salt agar	N/A	N/A	N/A	N/A	N/A	N/A	Bright yellow	N/A	N/A
Motility	+	-	-	+	+	+	-	-	-
Catalase Test	+	+	+	+	+	+	+	+	_
Coagulase	N/A	N/A	N/A	N/A	N/A	N/A	+	_	N/A
Citrate	-	+	+	-	+	+	N/A	N/A	N/A
Oxidase	-	-	-	-	+	-	-	-	-
Indole	+	-	+	-	-	+/-	-	-	-
Methyl red	+	-	-	+	-	+	+	+	_
Vogus Proskauer	_	+	+	+/-	+	_	+	N/A	N/A
Novobiocin	N/A	N/A	N/A	N/A	N/A	N/A	S	R	N/A
Growth on TSI									
Slant	Y	Y	Y	R	R	R	N/A	N/A	N/A
Butt	Y	Y	Y	Y	R	Y	N/A	N/A	N/A
H_2S	-	-	-	+	-	-	N/A	N/A	N/A
Gas production	+	++	++	+	-	+	N/A	N/A	N/A

N/A-Not applicable, R- Resistant. S – Sensitive, TSI- Triple sugar iron, EC – Escherichia coli, KP- Klebsiella pneumonia, KO- K. oxytoca, PM- Proteus mirabilis , PA- Pseudomonas aeruginosa, CI- Citrobacter spp, SA- Staphylococcus aureus, SS- S. saprophyticus , EF- Enterococcus faecalis

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Table 2: Antibiotics discs and discs contents susceptibility/resistance for clinical samples:

Group	Antibiotics	Symbol	Disc contents
Penicillins	Ampicillin	AMP	10 µg
	Amoxicillin	AMX	25mg
Cephalosporins	Ceftazidime	CEP	30 µg
	Cefpodoxime	CPD	10 µg
	Cephalexin	CN	10 µg
	Cephotaxime	CA	30 µg
Quinolones	Ciprofloxacin	CIP	10 µg
	Norfloxacin	NX	10 µg
	Ofloxacin	OF	5 µg
	Nalidixic acid	NA	30 µg
Aminoglycosides	Gentamycin	GEN	10 µg
	Amikacin	AK	30 µg
Macrolides	Eryhtromycin	E	10 µg
Sulphonamides	Cotrimoxazole	COT	1.25/23.75 µg
Glycopeptides	Vancomycin	VA	30 µg
Tetracycline	Tetracycline	TE	30 µg
Other antibacterial agent	Nitrofurantoin	NIT	300 µg
	Rifampicin	RIF	5C

Table 3: Microbial Pattern of Multidrug Resistance Isolates

Organism	Sample	Total isolates	Multidrug resistant		Total % of MDR isolates
			No.	%	
E.coli	Urine	45	18	40.0	65.0
	Pus	4	1	25.0	
Klebsiella pneumoniae	Urine	9	4	44.4	94.4
	Pus	2	1	50.0	
Pseudomonas aeruginosa	Urine	3	1	33.3	58.3
	Pus	4	1	25.0	
Staphylococcus aureus	Urine	7	2	28.5	54.5
	Pus	27	7	25.9	
Enterococcus faecalis	Urine	2	1	50.0	50.0
	Pus	-	-	-	
Total Multidrug resistance isolates		103	36	34.9	

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Table 4: Percentage of Susceptibility of Gram Positive Multidrug resistance Organism towards the Antibiotics

Antibiotic	Susceptibility		Resistant	
	No.	%	No.	%
Ofloxacin	10	100.0	-	-
Vancomycin	10	100.0	-	-
Ciprofloxacin	10	100.0	-	-
Gentamycin	6	60.0	4	40.0
Tetracycline	5	50.0	5	50.0
Ceftazidine	4	40.0	6	60.0
Erythromycine	4	40.0	6	60.0
Cefotaxime	4	40.0	6	60.0
Chloramphenicol	3	30.0	7	70.0
Amoxicillin	2	20.0	8	80.0
Ampicillin	2	20.0	8	80.0
Norfloxacin (urine)	1	10.0	9	90.0
Nitrofurantoin(urine)	-	-	10	100.0

Table 5: Percentage of Susceptibility of Gram-Negative Multidrug resistance Organism towards the Antibiotics

Antibiotic	Susceptibility		Resistant	
	No.	%	No.	%
Ofloxacin	24	92.3	2	7.6
Amikacin	23	88.4	3	11.5
Nitrofurantoin	12	46.1	14	53.8
Gentamycin	11	42.3	15	57.6
Ceftazidine	6	23.0	20	76.9
Cefotaxime	5	19.2	21	80.7
Chloramphenicol	4	15.3	22	84.6
Norfloxacin	3	11.5	23	88.4
Nalidixic acid	2	7.6	24	92.3
Ciprofloxacin	1	3.8	25	96.1
Cefpodoxime	-	-	100	100.0
Amoxicillin	-	-	100	
Cotrimoxazole	-	-	100	
Ampicillin	-	-	100	

It was found that 19 (65.0 %) E.coli were multidrug resistant out of 49 (47.5) isolates isolated from 281 samples (including Urine, Pus,), 9 (54.5%) Staphylococcus aureus were multidrug resistant out of 34 (33.0%) isolates. Similarly 5 (94.4%) Klebsiella pneumonia out of 11(10.6%) isolates, 2(58.3%) Pseudomonas aeruginosa out of 7(6.7%) isolates, and 1(50.0%) Enterococcus faecalis out of 2(2.0%) isolates were Multidrug resistance

Table 4 shows the Percentage of Susceptibility of Gram Positive Multidrug resistance Organism towards the antibiotics. The susceptibility pattern showed that, this multidrug-resistant gram positive isolates were cent percent sensitive to Ofloxacin, Vancomycin, Ciprofloxacin and cent percent resistant to Nitrofurantoin (urine). These isolates were 9 (90.0%) resistant to Norfloxacin, 8(80.0%) resistant to Ampicillin and Amoxicillin, 7 (70.0%) resistant

to Chloramphenicol, 6 (60.0%) resistant to Cefotaxime , Erythromycin, and Ceftazidine, 5 (50%) resistant to Tetracycline and 4 (40%) resistant to Gentamycin.

Table 5 shows the percentage of susceptibility of Gram –negative multidrug resistance organism towards the antibiotics. The susceptibility pattern showed that, these multidrug-resistant gram negative isolates were cent percent resistant to Cotrimoxazole, Cefpodoxime, Ampicillin and Ofloxacin. These isolates were 25 (96.1%) resistant to Ciprofloxacin, 24 (92.3%) to Nalidixic acid 23 (88.4%) to Norfloxacin, 22 (84.6%) to Chloramphenicol, 21 (80.7%) to Cefotaxime, 20 (76.9%) to Ceftazidine, 15 (57.6%) to Gentamycin, 14 (53.8%) to Nitrofurantoin, 3 (11.5%) to Amikacin and 2 (7.6%) to Amoxicillin.

DISCUSSION

Infections caused by resistant pathogens result in significant morbidity and mortality, and contribute to escalating healthcare costs worldwide. Despite the availability of newer antibiotics, emerging antimicrobial resistance has become an increasing problem in many pathogens throughout the world [17].

Of the total 227 urine samples processed, 76 (33.4%) showed significant growth of which, 26 (34.2%) were found to be multidrug resistance. In a study carried out at National Public Health Lab (NPHL), only237/1402)16.8 (%urine samples showed significant growth of which 64.6%] isolates showed multidrug resistance24 Of the total.227 mplesurine sa, only76)33.4 (%samples showed single type growth ,28)12.3 (%samples showed mixed growth whereas 123 (54.1%) urine samples showed no growth . In similar studies carried out in different parts of Nepal showed low number growth positivity. The low growth positive rate observed in our study might be due to inclusion of every urine samples for culture regardless of their illness and symptoms ,the referral of all the patients seeking intervention regarding problems of urinary tract to urine culture, prior use of the antibiotics the, or the possible presence of the fastidious bacteria] 10, 6, 24] .

During the study the commonest organism isolated from Urine sample was Escherichia coli 45 (59.2%) followed by Klebsiella spp 11(14.4%) Staphylococcus aureus 7(9.2%), Proteus mirabilis 6(6.5%), Pseudomonas aeruginosa 3 (3.9%), Enterococcus faecalis 2 (2.6%), Staphylococcus saprophyticus (2.6%), Citrobacter spp 1(1.3%).These results ressembled the]outcomes of previous studies by18 ,6.[

E. coli have special virulent properties contributing to their being a major uropathogen throughout the world. E. coli can bind to the Glycoconjugate receptor (Gal alpha1-4 Gal) of the uroepithelial cells of human urinary tract such that it can initiate infection itself [9].

The culture of the pus sample showed the positive growth of 37 (68.5%) and negative growth of 17 (31.4%). In our study, the most frequently isolated organism from wound specimen was S. aureus (72.9%), the only Gram positive isolate then E.coli and P. aeruginosa (10.8%) followed by K. pneumoniae (5.4%). Bomjan (2005) also done microbiological analysis of

pus/ wound infection and reported frequently isolated organism from wound specimen was Staphylococcus aureus (70.9%), E.coli (16.1%), Pseudomonas aeruginosa (9.6%) and Klebsiella pneumonia (3.2%).

Bacterial resistance to antimicrobial agent is a major public health problem in many tropical countries [14, 2]. There is not a specific definition of multidrug resistance. Some define MDR as resistance to usually employed drug and some define it as resistance to two or more drugs to which bacteria are usually susceptible [14]. In our study the organism is considered as MDR, when it is resistant to two or more groups of antibiotics.

It was found that 36 multidrug resistant isolates isolated from 281 samples where 19 (65%) out of 49 were E.coli. 5 (94.5%) out of 11 were Klebsiella pneumoniae, 2 (58.3 %) out of 7 were Pseudomonas aeruginosa, 9 (54.5%) out of 34 Staphylococcus aureus, 1(50.5) out of 2 Enterococcus faecalis.

The susceptibility pattern showed that, these multi drug-resistant Gram Positive isolates were cent percent sensitive to Ofloxacin, Vancomycin, Ciprofloxacin and cent percent resistant to Nitrofurantoin (urine). The high susceptibility to Ofloxacin, Ciprofloxacin andVancomycin is a welcome relief since it is an indication of effectiveness of the antibiotics against that bacteria. This study is an agreement with Nkang et al., (2007)[20]. These isolates were 90% resistant to Norfloxacin, 80% resistant to Ampicillin and Amoxicillin, 70% resistant to Chloramphenicol, 60% resistant to Cefotaxime, Erythromycin, and Ceftazidine 50% resistant to Tetracycline and 40% resistant to Gentamycin.

From the study it was found the high occurrence of the Nitrofurantoin resistant gram – positive organism. This may be due to the modification or derivation of the recurring pathway strategies of enzymatic activity, altered target or decreased uptake [7].

The susceptibility pattern showed that, these multi drug-resistant gram negative isolates were cent percent resistant to Cotrimoxazole, Cefpodoxime, Ampicillin and Amoxicillin. These isolates were 96.1% resistant to Ciprofloxacin, 92.3% to Nalidixic acid 88.4% to Norfloxacin, 84.6% to Chloramphenicol, 80.7% to Cefotaxime, 76.9% to Ceftazidine, 57.6% to Gentamycin, 53.8% to Nitrofurantoin, 11.5% to Amikacin and 7.6% to Ofloxacin.

In case of gram-negative multidrug resistant organism high occurrence of the Ampicillin resistant organism. This may be due to the production of Penicillinase. Bomjan (2005)[8] also reported high level of Ampicillin resistant organism from clinical isolates. Bermer- Melchior et al., (1995) [7] also reported high level of penicillinase producer E.coli (92.1%) from clinical isolates.

Resistance to Amoxicillin, Cotrimoxazole and Cephalexin is most frequently mediated by either decreased uptake or accumulation or by production of an altered target [7].

High rate of drug resistance were found in most of the isolates studied. In developing countries like Nepal self medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates. Since patient only think of going to the hospitals when they are unable to treat themselves. Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioner can also lead to emergence of resistance in bacteria. Expired antibiotics, self-medication counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates [23].

Because of these high incidences of antibiotic refractiveness by infectious bacteria, many people, including even the urban dwellers, have turned to traditional herbs to seek for succor [15]. Development of multi-drug resistance by the bacterium has further complicated the problem. Antibiotic resistance is further accelerated due to irrational use of antibiotics and over-the-counter purchase attitude by the populace, which is a very common phenomenon in developing [15].

Conclusion

Multi-drug resistance pattern among clinical isolates (urine and pus) was high i.e. 65% in E. coli 94.4% in Klebsiella pneumoniae, 58.3% in Pseudomonas aeruginosa, 54.5% in Staphylococcus aureus and 50.0% in Enterococcus faecalis, still remain the most frequently isolated pathogens with high level of multidrug resistance . Norfloxacin had the lowest sensitivity towards Gram-positive multidrug resistant isolates isolated from urine sample and Ciprofloxacin had the lowest sensitivity towards the multidrug resistant Gram-negative isolates isolated from urine and pus samples.

Multidrug resistance among bacterial pathogens is a major health problem in Nepal that thwarts the management of several infectious diseases and compromises therapy. Determining the multidrug resistance patterns of the disease causing organisms will enable health institutions to restrict the use of antimicrobials and take active measures in preventing the spread of drug resistance in hospitals.

However, the judicious use of antibiotics by health workers and efforts to control procurement and use of antibiotics officially in all location in Nepal will probably help to limit the increasing rates of multidrug resistance in pathogens. Thus, controlling antibotic resistant bacteria and subsequent infections more efficiently necessitates the prudent and responsible use of antibiotics.

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