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Full Length Research Paper

Epidemiology and antibiotic susceptibility of bacterial isolates from Northern Pakistan

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This study was conducted in Khyber Teaching Hospital, to evaluate antimicrobial susceptibility pattern among pathogens isolated in a tertiary care hospital. Specimens were collected in sterile containers and cultured for a period ranging from 24-48 h at 37℃ aerobically, using appropriate bacteriological isolation media. Isolates were identified using Gram staining and biochemical reactions. Antibiotic susceptibility was determined by standard disc diffusion (Kirby-Bauer) Method. Among gram-negative rods*, Escherichia coli* was the predominant pathogen 315 (34.5%), followed by *Citrobacter* spp. 124 (13.6%), *Enterobacter* spp. 80 (8.8%), *Pseudomonas* spp. 51 (5.6%) *Proteus* spp. 35 (3.8%) *Morganella morganii* 16 (1.8%) and *Salmonella* spp. 8 (0.9%). *Staphylococcus aureus* was the predominant gram positive pathogen 196 (21.4%) followed by *Staph epidermidis* 32 (3.5%) and Streptococci 7 (0.7%). *Pseudomonas* spp. and *E. coli* were the most common pathogens recovered from mixed polymicrobial growth. Out of 16 antimicrobial agents tested, only 6 were effective, inhibiting growth of >50% of the strains of each isolated pathogen. The rest of the 10 antimicrobial agents tested were ineffective to inhibit growth of even 50% of the strains of recovered pathogens. Although the susceptibility pattern to some of the antibiotics such as Imipenem and Meropenem is very good but they are very expensive. The cheaper and affordable antibiotics were least effective. The increasing level of resistance in microorganisms to these cheaper low cost agents is a matter of significant concern.

Key words: Antimicrobial susceptibility, antibiotic resistance, bacterial isolates.

INTRODUCTION

The prevalence of antibiotic resistance in pathogens is increasing worldwide. The increasing resistance to antimicrobial agents is a cause for concern. Infections produced by microorganisms challenge antimicrobial therapy with the steady emergence of antibiotic resistant strains. Generally, pathogens isolated from hospital acquired infections (HAI) are more resistant when compared with pathogens isolated from community acquired infections (Jawad et al., 2005). Certain hospital associated infections are more common, that is, urinary tract infections (UTI), respiratory infections (RTI), surgical site infections (SSI), and blood stream infections. Infection caused by multi-drug resistance bacteria is a serious problem for especially intensive care unit patients (ICU) throughout the world (Jazayeri and Irajian, 2009). Prolonged hospitalization, as a result of severe underlying disease, mechanical ventilation, indwelling urinary catheters, and previous antimicrobial therapy are all key factors in predisposing to HAI (Plowman, 1997). Common factors contributing to the development of HAI

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Abbreviations: HAI, Hospital acquired infections; **UTI,** urinary tract infections; **RTI,** respiratory infections; **SSI,** surgical site infections; **ICU,** intensive care unit patients; **ARIs,** acute respiratory tract infections; **ENT,** ear, nose and throat; **C/S,** culture and sensitivity; **FATA,** Federally administered tribal areas; **cfus,** colony forming units; **CLSI,** clinical and laboratory standards institute.

are the patients' health status, the physical environment and those that result from clinical intervention that increase the patient's inherent risk (Barie, 2002). If preventive measures are not followed properly, the patients, medical and paramedical staff may also contribute to HAI (Jawad et al., 1994).

Urinary tract infection is one of the most common infections encountered in the medical practice (Jawad et al., 2003). The mortality rate associated with multi-drug Gram-negative enteric bacteria in these patients is high in some intensive care units (Plowman, 1997). Acute respiratory tract infections (ARIs) are the leading cause of death in young children in Pakistan, responsible for 20-30% of all child deaths under age 5 years. Widespread antimicrobial resistance amongst respiratory pathogens to commonly used, affordable antibiotics has made the treatment of ARI in developing countries very difficult (Zaidi, 2003; Khan et al., 2004). Typhoid fever is endemic in developing countries including Pakistan. Emergence of multi-drug resistant *Salmonella. typhi* and *Salmonella paratyphi* has added to this problem. The incidence of multi-drug resistance *Salmonella typhi* has increased in the last few years (Butt et al., 2000). Non-typhoidal salmonellae are the common etiological agents in diarrhoeal diseases which are resistant to quinolones and pose a serious therapeutic challenge (Panhotra et al., 2004).

Antibiotics are unnecessarily prescribed for all sorts of upper respiratory problems, for example, common cold. Muco-purulent rhinitis is associated with common cold. The colour is due to high eosinophil count in secretions for which antibiotics are generally not effective, guidelines specifically recommend against using antibiotics to treat the rhinitis (Aroll, 2002). One side effect of inappropriate use of antibiotics is the development of antibiotic resistance in microorganisms. This complicates management, producing more health problems, increase financial burden on patients ultimately affecting economy of the nation.

This study was initiated to identify the range of etiological agents of nosocomial as well as community acquired infections and their susceptibility pattern to various commonly used antimicrobial agents in this region. This study is a step towards planning hospital antibiotic policy, which will provide guidelines to clinicians in prescribing most appropriate antimicrobial agents in the absence of facilities for culture/sensitivity. This will help in controlling the emergence of drug resistance in bacteria in this particular region.

MATERIALS AND METHODS

This study was conducted at the microbiology department of Khyber Teaching Hospital Peshawar from October 2003 to January 2005 to investigate the varieties of pathogens responsible for different kinds of infections and their antibiotic susceptibility pattern. It is a 1200 beds tertiary care hospital with the facilities for many disciplines of medicine, that is, general medicine, ICU, general surgery, gynaecology, orthopaedic, ear, nose and throat (ENT),eye, pediatrics, nephrology, pulmonology, cardiology etc.

A total of 3108 specimens of urine, stool, pus, sputum, blood and body fluids were studied. The specimens for culture and sensitivity (C/S) test were received both from admitted patients and outdoor patients. Fresh specimens were collected after due precautions were taken. Urine was collected in a sterilized container, pus through disposable diagnostic swabs, fluids in sterilized disposable syringes, sputum in containers and blood in the nutrient broth medium. The patients came from different parts of Khyber Pakhtunkhwa and Federally Administered Tribal Areas (FATA) adjoining Afghanistan Boarder on the north-west side, including cities, towns, and villages.

Only those specimens collected in containers provided from the microbiology laboratory and taken freshly, were included in the study. Exclusion criteria were sample from outside and non fresh specimen. In the case of urine, if colony count was $>10^5$ colony forming units (cfus) growth was considered significant, sensitivity was recorded.

The specimens were cultured by inoculating onto blood agar and MacConkey agar, using a calibrated wire loop. After 24 h incubation at 37℃ in air (or in an anaerobic jars for some specim ens), these plates were examined and colonies counted. Isolated bacteria were identified by Gram staining, morphological appearance and biochemical tests. Pathogenic bacteria were identified with the help of various sugar fermentation tests.

Susceptibility was determined by standard disc diffusion using Kirby-Bauer method following Clinical and Laboratory Standards Institute (CLSI) guidelines. On the surface of agar, lawn of bacteria was made using cell suspension of test bacteria. Commercially available antimicrobial containing discs (Oxoid, UK) were placed aseptically on the surface of agar plate with the help of a sterile forceps at well-spaced interval. A total of 16 agents were selected from different antimicrobial classes: cephalosporins included cephradine, ceftazidime, ceftriaxone, ceftizoxime, cefixime and sulzone (cefoperazone + sulbactam). quinolones included enoxacin, ciprofloxacin, sparfloxacin and moxifloxacin. From macrolide group erythromycin and clarithromycin and from carbapenem imipenem, meropenem were included. One agent each from aminoglycosides and penicillins, that is, amikacin and tazocin (piperacillin + tazobactam), respectively, were included. Once applied, each disc was gently pressed to ensure its firm contact with the agar surface. These Petri dishes were incubated aerobically at 37℃ for 18 h.

The plates were examined for zone of inhibition. The susceptibility of organism was determined using CLSI recommendations. Accordingly, the results were recorded as sensitive (S), intermediate (I) and resistant (R) respectively for each antimicrobial agent and microorganism. Antimicrobial agents were divided into two groups on the basis of sensitivity to them, that is, Group A, >50% of the strains of isolates and Group B, <50% of the strains of the isolate were sensitive to antimicrobial agents tested.

A proforma was prepared which included various parameters for the study. Patient's demographic data included name, age, sex, address, etc. while the lab information included names of the isolated pathogens and the susceptibility pattern of these microorganisms to all the range of antimicrobial agents tested in this study. The data was transferred from proforma to a computer worksheet and evaluated.

RESULTS

A total of 3108 patients were included in this study during the period from October 2003 to January 2005. Age range was 1 day-75 year, mean 14 years. Male patients were 534 (58.5%) and females were 379 (41.5%) and

Table 1. Distribution of bacteria isolated (n=913).

S/N	Bacterial isolate	Number	Percentage
1	*E. coli*	315	34.5
2	*Citrobacter* spp.	124	13.6
3	*Enterobacter* spp.	80	8.8
4	*Pseudomonas* spp.	51	5.6
5	*Proteus* spp.	35	3.8
6	*Salmonella* spp.	8	0.9
7	*Morganella* spp.	16	1.8
8	*Staph aureus*	196	21.4
9	*Staph epidermidis*	32	3.5
10	Streptococci	7	0.7
11	Mixed growth	41	4.5
12	Miscellaneous	8	0.9
	Total	913	100

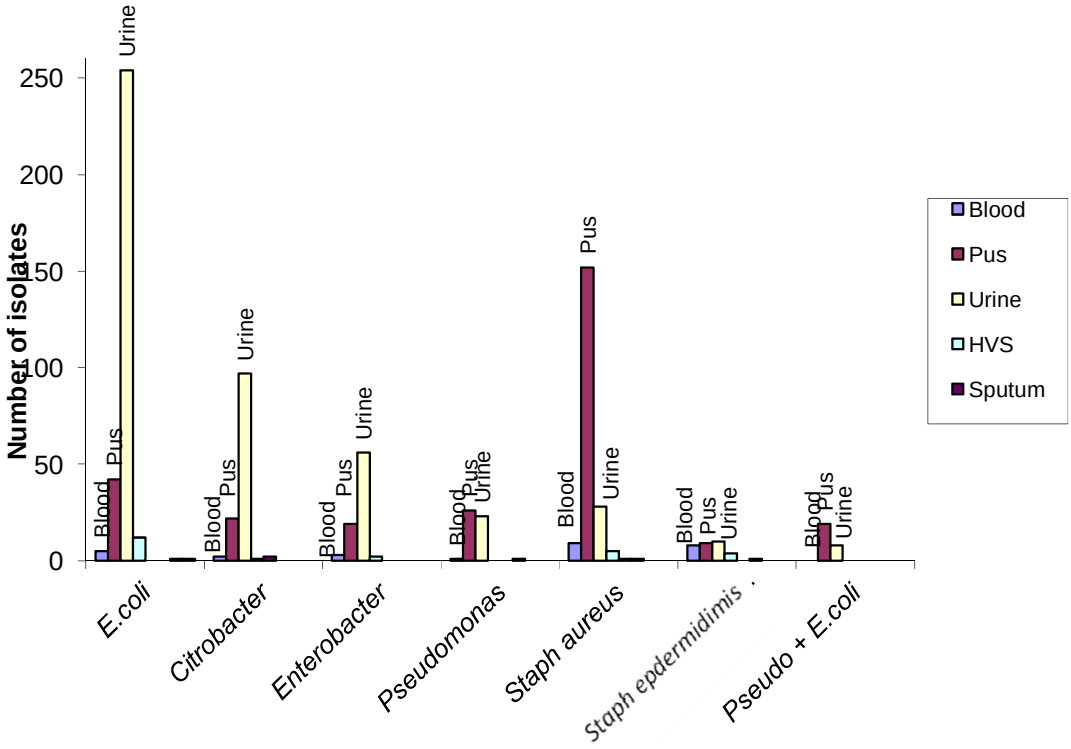

Figure 1. Bacterial isolates recovered from various specimens in this study.

male to female ratio was 1.41:1. Growth was yielded in 913 specimens (29.4%).

Among gram-negative rods the relative frequency of *E. coli* was very high (34.5%) followed by *Citrobacter* spp. (13.6%) and *Enterobacter* spp. (8.8%). Among Gram positive cocci, *Staph aureus* (21.4%) was the most common pathogen isolated, followed by *Staph epidermidis* (3.5%), (Table 1). Specimens from which these pathogens were isolated included urine, pus, blood, sterile body fluids, sputum etc. (Figure 1). Isolates were obtained mainly from urine specimens. *E. coli* was the main organism in it followed by *Citrobacter* spp., *Enterobacter* spp., *Pseudomonas* spp., *Staph aureus* and *Staph epidermidis* in decreasing order, while *Staph aureus* was mainly cultured in pus.

Some mixed growth was also yielded in this study. *Pseudomonas* spp. was present in all the three different polymicrobial mixed growths along with *E. coli* (65.9%),

Table 2. Antimicrobial susceptibility (Kirby-Bauer method) of multiple aerobic isolates from patients during prospective study in Khyber Teaching Hospital. Percentage of isolates sensitive to the indicated antibiotic used in the study.

Isolates (n)	AK	TZP	IMP	MEM	SCF	V	CAZ	CRO	ZOX	CFM	ER	KLR	ENX	CIP	SPX	MXF
E. coli (315)	51.1	54.3	95.6	74.9	66.3	7.1	32	36.8	32.7	25.4	NT	NT	46.7	39	48.3	40.6
Enterobacter spp. (80)	42.5	38.8	83.8	63.8	48.8	1.3	10	13.8	11.3	2.5	NT	NT	37.5	35	48.8	41.3
Citrobacter spp. (124)	40.3	41.1	97.6	74.2	53.2	2.42	13	22.6	9.7	8.9	NT	NT	52.4	45.2	64.5	48.4
Pseudomonas spp. (51)	41.2	35.3	90.2	76.5	66.7	0	21.6	19.6	23.5	0	NT	NT	37.2	27.4	17.6	56.9
Proteus spp. (35)	34.3	48.6	94.3	77.1	71.4	5.7	22.9	45.7	22.9	8.6	NT	NT	37.1	34.3	48.6	42.9
Salmonella spp. (8)	75	75	87.5	87.5	75	25	37.5	50	37.5	37.5	NT	NT	62.5	50	50	50
Morganella morganii (16)	37.5	50	81.3	75	56.3	12.5	56.3	68.8	75	75	NT	NT	56.3	50	62.5	50
Pseudomonas spp.+ *E .coli* (27)	33.3	33.3	88.9	74	48.2	3.7	7.4	7.4	0	0	NT	NT	37	14.8	33.3	33.3
Staph aureus (196)	63.3	47.4	82.7	63.8	70.4	26	424	53.6	50	30.6	32.1	32.1	60.2	57.7	78.6	76.5
Staph epidermidis (32)	75	78.1	90.6	75	71.9	28.1	31.3	34.4	18.8	15.6	15.6	15.6	62.5	46.9	87.5	90.6
Streptococcus spp. (7)	14.3	71.4	42.9	71.4	71.4	28.6	0	14.3	14.3	0	0	0	28.6	14.3	42.6	42.6

Key: AK=Amikacin, TZP=Tazocin (Piperacillin/Tazobactam) IMP=Imipenem, MEM=Meropenem, SCF=Sulzone (Cefoperazone/ Sulbactam) V = Cephradine, CAZ = Ceftazidime, CRO = Ceftriaxone, ZOX = Ceftizoxime Sodium, CFM = Cefixime, ER = Erythromycin, KLR = Clarithromycin, ENX = Enoxacin, CIP = Ciprofloxacin, SPX = Sparfloxacin, MXF =Moxifloxacin, NT (Not Tested).

Staph aureus (24.3%) and with *Citrobacter* spp. (9.8%).

Table 2 shows percentage of sensitivity of isolates to 16 antibiotics tested in this study. The sensitivity pattern of Imipenem, Meropenem, Tazocin and Sulzone was better for all organisms compared to all other classes of antibiotics tested, that is, quinolones or cephalosporins. Quinolones were more effective against *Staph epidermidis, Staph aureus, Citrobacter* spp., *Salmonella* spp., Streptococci and *Morganella morganii* compared to *E. coli* and *Proteus* spp. The least effective antimicrobial agents were macrolides: erythromycin and clarithromycin tested against staphylococci and streptococci. In cephalosporins group the least effective antimicrobial agents were Cephradine and Cefixime compared to Ceftraixone, Ceftizoxime and Ceftazidime.

Table 3 shows percentage of isolates resistant to 16 antimicrobial agents tested in study. Many organisms recorded high level of resistance to Cefixime (70-100%), Cephradine (60-98%) and Macrolides (60-85%) The resistance pattern by *Morganella* spp. and *Salmonella* spp. was comparatively low to Cefixime. High level of resistance was also recorded to quinolones in *E. coli* (≅50%), *Enterobacter* spp. (≅50%), *Citrobacter* spp. (≅40%) and *Pseudomonas* spp. (≅50%).

On the basis of cumulative sensitivity for all microorganisms to various antimicrobial agents tested (Table 4) these antimicrobial agents could be divided into two categories: Group A included imipenem, meropenem, cefoperazone/sulbactam, sparfloxacin, moxifloxacin, amikacin, and group b enoxacin, piperacillin + tazobactam, ciprofloxacin, ceftriaxone, ceftizoxime, erythromycin, clarithromycin, ceftazidime, cefixime and cephradine.

Table 4 shows cumulative sensitivity, intermediate and resistance pattern for all microorganisms to various antimicrobial agents. Highest level of resistance was recorded by all organisms to Cephradine (84.5%) and least resistance was recorded for Imipenem (6.9%).

DISCUSSION

In spite of the advancement in the prevention and treatment of infectious diseases, microorganisms still present continuous challenge to the antimicrobial chemotherapy with steady emergence of antibiotic resistant strains. These are responsible for worsening the living conditions of millions of people around the world (Jones et al., 2010). Antibiotic resistance has become a serious problem in both the developed and developing countries such as Pakistan. The cost of broad spectrum effective antibiotics is very high and out of the reach of ordinary middle class citizen in a developing country like Pakistan. This has complicated the situation and help in

Table 3. Antimicrobial susceptibility (Kirby-Bauer Method) of common aerobic isolates from patients during prospective study in Khyber Teaching Hospital. Percentage of isolates resistant to the indicated antimicrobial agents used in the study.

Organism (No of isolates)	AK	TZP	IPM	MEM	SCF	V	CAZ	CRO	ZOX	CFM	ER	KLR	ENX	CIP	SPX	MXF
E. coli (315)	40.32	33.1	3.8	197	22.9	86.6	61.6	60.3	63.2	70.8	NT	NT	52.39	57.5	48.9	53
Enterobacter spp. (80)	33.75	36.3	6.3	288	35	98.8	83.8	83.8	86.3	95	NT	NT	57.5	58.8	41.3	47.5
Citrobacter spp. (124)	36.3	42	1.6	15.3	32.3	93.6	79	72.6	89.5	89.5	NT	NT	46.8	49.2	28.2	42.7
Pseudomonas spp. (51)	45.1	62.8	3.9	21.6	29.4	98	76.5	76.5	72.6	98	NT	NT	54.9	51	60.1	41.2
Proteus spp. (35)	57.15	51.4	5.7	22.9	28.6	94.3	77.2	54.3	77.2	91.4	NT	NT	57.2	60	45.8	51.4
Salmonella spp. (8)	25	12.5	12.5	12.5	25	62.5	37.5	50	50	37.5	NT	NT	12.5	50	37.5	25
Morganella morganii (16)	62.5	43.8	12.5	18.8	31.3	81.3	25	25	12.5	18.8	NT	NT	37.5	43.8	25	43.8
Pseudomonas spp.+ *E .coli* (27)	55.56	44.5	7.4	22.2	44.5	92.6	92.6	92.6	100	100	NT	NT	59.3	81.5	55.6	59.3
Staph aureus (196)	18.37	35.2	13.8	23.5	26.5	69.4	53	43.9	48.5	68.9	61.2	63.8	31.1	40.8	14.8	15.8
Staph epidermidis (32)	21.88	15.6	9.3	18.8	21.9	59.4	59.4	53.1	75	84.4	78.1	78.1	37.5	46.9	12.5	3.1
Streptococcus spp. (7)	57.15	28.6	57.2	0	0	71.43	100	85.7	71.4	100	85.7	85.7	71.4	71.4	42.6	42.6

Key: AK=Amikacin, TZP=Tazocin (Piperacillin/Tazobactam) IMP=Imipenem, MEM=Meropenem, SCF=Sulzone (Cefoperazone/Sulbactam) V = Cephradine, CAZ = Ceftazidime, CRO = Ceftriaxone, ZOX = Ceftizoxime Sodium, CFM = Cefixime, ER = Erythromycin, KLR = Clarithromycin, ENX = Enoxacin, CIP = Ciprofloxacin, SPX = Sparfloxacin, MXF = Moxifloxacin, NT (Not Tested).

Table 4. Cumulative susceptibility patterns of different pathogens to antimicrobial agents used during this prospective study.

Antimicrobial agent	Total No of isolates	Sensitive (%)	Intermediate (%)	Resistant (%)
Amikacin	891	448 (50.2)	127 (14.3)	316 (35.5)
Tazobactam/Piperacillin	891	434 (48.7)	126 (14.2)	331 (37.2)
Imipenem	891	806 (90.5)	23 (2.6)	62 (7.0)
Meropenem	891	638 (71.6)	68 (7.6)	185 (20.8)
Cefoperazone/Sulbactam	891	567 (63.6)	81 (9.1)	243 (27.3)
Cephradine	891	96 (10.8)	42 (4.7)	753 (84.5)
Ceftazidime	891	251 (28.2)	53 (6.0)	587 (65.9)
Ceftraixone	891	315 (35.4)	29 (3.2)	547 (61.4)
Cefizoxime	891	264 (29.6)	27 (3.0)	600 (67.3)
Cefixime	891	176 (19.8)	21 (2.4)	694 (77.9)
Erythromycin	235	68 (28.9)	16 (6.8)	151 (64.3)
Clarithromycin	235	68 (28.9)	11 (4.7)	156 (66.4)
Enoxacin	891	438 (49.2)	35 (3.9)	418 (46.9)
Ciprofloxacin	891	378 (42.4)	46 (5.2)	467 (52.4)
Sparfloxacin	891	505 (56.7)	59 (6.6)	327 (36.7)
Moxifloxacin	891	468 (52.5)	66 (7.4)	357 (40.0)

development of more resistant pathogens.

The problem of antibiotic resistance is worsened, when the consumption of antibiotics is increased (Oteo et al., 2009), when antibiotics are used inappropriately or prescribed by the physicians without the availability of proper sensitivity report. Antibiotic resistance is one of the most pressing health problems. It can cause significant danger and sufferings for people who have common infections that once were easily treatable with simple antibiotics. The outcomes of antibiotic resistance are long lasting illnesses leading to high level of morbidity and mortality.

E. coli, *Citrobacter* spp*.*, *Enterobacter* spp*.*, *Pseudomonas* spp*.*, *Morganella morganii, Salmonella* spp*.* and Gram-positive bacteria, that is, *Staphylococcus aureus* and *Staphylococcus epidermidis* and Streptococci, have been isolated as pathogens in this study (Figure 1). In other studies coagulase negative gram-positive cocci, were the predominant pathogens (26%), followed by coagulase positive *S. aureus* (8%) (Butt et al., 2004). The spectrum of isolates among febrile neutropenic patients in our population appears to be shifting towards gram-positive microorganisms (Butt et al., 2004). Our previous experience also supports the current study to some extent (Jawad et al., 2003). The findings of our current study are similar to that of Hussain (2002).

Out of 16 antimicrobial agents only 6 were effective, inhibiting growth of >50 of the strains of each isolated pathogen (Group A) using cumulative mean for all organisms. Our findings are similar to other studies (Mahmood et al., 2002) where they have observed that more than 90% of the gram-negative isolates were sensitive to imipenem/meropenem and piperacillin + tazobactam. But the cost of a full course of such antibiotics is out of the range of a middle class citizen of this country. The rest of the 10 antimicrobial agents tested (Group B) were ineffective to inhibit growth of even 50% of the strains of recovered pathogens. Majority of the gram-positive isolates in the present study were resistant to macrolide group: erythromycin (64.3%) and clarithromycin (66.4%). These results should be kept in mind by local clinicians when infections suspected as a result of Gram positive bacteria are treated. Macrolide are widely used for the empirical treatment of lower RTIs due to their activity against *Streptococcus pneumoniae* and other common respiratory pathogens. However, the prevalence of pneumococcal resistance to macrolide is increasing all around the world (Marrie, 2004).

In our country, multidrug-resistance in isolates of *E. coli* is much higher to third generation cephalosporins and quinolones (Iqbal et al., 2002; Karlowsky et al., 2006). The consumption of quinolones has been correlated with increasing resistance among hospitalized patients (Sabir et al., 2004). *E. coli* has been found to be highly susceptible to ofloxacin and norfloxacin (Jazayeri and Irajian, 2009) but highly resistance to ampicillin and cotrimoxazole (Hussain, 2002). This is in contrast to our study where level of resistance in *E. coli* was high to quinolones (Table 4). This has also been reported from other regions of the world, especially Spain in which 22% of the *E. coli* isolates were ciprofloxacin resistant (Jones et al., 2010).

When cephalosporins were combined with Sulbactam, the inhibiting power was drastically increased, as is the case of Sulzone from Group A (Table 4). This shows that combinations of antimicrobial agents are more effective and many more similar combinations must be tested and applied in clinical trials to control complicated polymicrobial infections not usually covered by ordinary antimicrobial agents.

The major contributing factors to increasing level of resistance to commonly used antibiotics in these isolates in this region may be free availability of antibiotics over the counter, prescription of antibiotics without susceptibility report, de-escalation according to clinical course etc. Further research at molecular level is required on those resistant genes carried by these pathogens and the sequences may be compared with similar genes reported from other parts of the World.

Conclusion

Bacterial infections are an important source of co-morbidity in the resource limited settings. The emergence of resistant microorganisms is a global problem, not restricted to a particular region. Out of 16 antimicrobial agents tested, only 6 were effective. Although the susceptibility pattern to some of the antibiotics such as imipenem and meropenem is very good but they are very expensive. The cheaper and affordable antibiotics were least effective. The increasing level of resistance in microorganisms to these cheaper low cost agents is a matter of significant concern. The results of present study have shown that the important and essential strategies for controlling the problem of multi-drug resistant organisms in the community as well as hospitals infection should be directed towards continuous monitoring of these resistant organisms, and the avoidance of continued or over use of any antibiotics over a long period of time. Appropriate preventive measure of this problem is the proper record keeping (surveillance) of resistant organisms, in the hospital and community acquired infections. Clinicians should be cognizant of the most common organisms affecting patients, and of their local susceptibilities, when treating empirically bacterial infections.

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