PREVALENCE AND B-LACTAMASE PRODUCING BACTERIAL ISOLATES IN A TEACHING HOSPITAL IN PESHAWAR, PAKISTAN: A FOUR YEAR STUDY

JAMSHAID ALI KHAN, ZAFAR IQBAL, LIAQAT ALI* AND ABBAS KHAN**

Department of Pharmacy University of Peshawar, Peshawar, Pakistan
**Postgraduate Medical Institute, Hayatabad Medical Complex, Peshawar*
***Drug Regulatory Authority, NWFP*

ABSTRACT

Prevalence of microorganisms was studies in clinical samples of various body fluids (n=12259), collected from patients at Post graduate Medical Institute Hayatabad Medical Complex (PGMI, HMC) Peshawar. Only 34.90% of samples exhibited growth, 36.25% of these isolates were Gram positive and 63.75% were Gram negative bacteria. *E. coli* was the most prevalent organism (39.45%) followed by *S. aureus* (32.23%), *Proteus spp.* (9.23%), *Pseudomonas spp.* (6.54%), *Streptococuss spp.* (3.51%), *Acinetobacter spp.* (2.66%), *Citrobacter spp.* (2.8%), *Providencia spp.* (2.2%) and miscellaneous bacteria (1.38%). The growth of bacteria was high in pus samples (44.03%) followed by urine (38.21%), high vaginal swab (HVS) (8.58%), cerebrospinal fluid (CSF) (1.96%), blood (3.39%) and miscellaneous samples (3.83%). *E. coli* and *S. aureus* were the most prevalent organism in urine (56.57%) and pus samples (44.02%), respectively. The frequency of *E. coli* (61.76%) was high in samples collected from female patients and percentage of the infections caused by the *S. aureus* in male and female patients was 47.9 and 52.1, respectively. During the study period, frequency of the *E. coli* was high during April to October and the prevalence of *S. aureus* was very common during March to October. Consistent but insignificant increase in the β-lactamase producing *S. aureus* and *E. coli* was observed throughout the period of the study. However, increase in the β-lactamase producing *S. aureus* and *E. coli* was above 80%. The prevalence of extended spectrum β- lactamase (ESBL) in *E. coli* was increased from 13.85% in year 2000 to 22.66% in year 2003.

The increasing in prevalence of microorganism, particularly of β-lactamase producing *E. coli* and *S. aureus* and ESLB is alarming situation. Various measured like prescribing and patient compliance are required to control the increase in the prevalence of microorganism.

Keywords: β-lactamases, prevalence, *S. aureus*, *E. coli*. pus, urine.

INTRODUCTION

Nosocomial infections are the major cause of morbidity and mortality in hospitalized patient (Nunez *et al*, 2000; Burke, 2003). Hospitalization invariably results in replacement of indigenous microbes by similar microbes with increased antibiotic resistance (John *et al*, 2000). Changes in the types of pathogens isolated in severe infection might also affect resistance species inherently differing in antimicrobial susceptibility (Klastersky, 1998).

E. coli, S. aureus, Pseudomonas, Proteus, Streptococcus are the most abundant organism responsible for the nosocomial and community acquired infection (Pfaller *et al*, 1998) and the frequency of *E. coli* is high in nosocomial infections compared with community acquired infections (Bert *et al*, 2003; Erdinc *et al*., 2006). More than 50% urinary tract infections (UTI) are due to *E. coli* and also showed high prevalence in female UTI compared with the male UTI (Sobel and Kaye, 2000).

S. aureus has been know as one of the most versatile nosocomial and community acquired pathogen (Lowy, 1998) and is responsible for more than 80% of the supportive disease encountered infection. It is most prevalent in wound blood and urinary tract infection (Kelkar, 2002).

The β-lactamases, an enzyme responsible for the destruction of β-lactam antibiotics, reacts with the β-lactam bond and form an acyl enzyme intermediate, which undergoes rapid hydrolysis, and results in the loss of activity (Oliva, 2003). Extended spectrum of β-lactamases (ESBLs) has been identified in Entero-bacteriaceae from patients through out the world and high prevalence was found in long term care facilities (Mendelson *et al*., 2005). Admission to a nursing home, excessive antibiotics exposure, extended hospital stay, recent surgery, admission to an ICU and contaminated instrumentation have been identified as risk factors for the ESBL producing strains (Lucet, 1996).

The urgent need was emphasized to strengthen the

Corresponding author: Tel: 091-9216750, Fax: 091-9218131, e-mail: zafar_iqbal@upesh.edu.pk

microbiological and epidemiological capacities of health care workers world wide to prevent transmission of nosocomial infections and to prepare them to address the problem of the emergence of multiple drug resistance among various bacterial isolates (Richet *et al,* 2003). The prevalence studies of the microorganism are essential to identify the most pathogenic organisms and resistant strains that will help to limit the spread of the resistance strains and effective use of therapeutics agents. The present work emphasizes on the prevalence of various microorganisms responsible for nosocomial infections.

MATERIAL AND METHODS

Study location

The prevalence and identification of bacterial isolates and studies on β-lactamase producer were performed at Postgraduate Medical Institute (PGMI) Hayatabad Medical Complex (HMC), Peshawar and Dr. Raza Pharma (Pvt.) Limited, Peshawar (Pakistan). This hospital provides the health facilities not only to the local population but also to the other parts of the province and the immigrants from Afghanistan.

Sample processing

Samples were collected from patients visiting out door patient clinics (OPD) and those admitted in different wards of the hospital. The pus samples were directly inoculated on blood agar and MacConkey agar and incubated for 24 to 48 hours at $37^{o}\pm1^{o}C$. Urine samples were cultured on Cystine-Lactose-Electrolyte Deficient (CLED) medium and were incubated for 24 to 48 hours at $37^{o}\pm1^{o}C$. Then bacterial isolates were sub cultured on blood agar and MacConkey agar. Blood samples were processed in Brain Heart Infusion broth and isolates were sub cultured on Blood agar and MacConkey agar plates and incubated for 24 hours at $37^{o}\pm1^{o}C$. The samples of cerebrospinal fluids were inoculated on chocolate agar and incubated at $37^{o}\pm1^{o}C$ for up-to 48 hours. Samples showing conspicuous growth were sub cultured on blood agar and MacConkey agar plates and incubated at $37^{o}\pm1^{o}C$ for further 24 hours. High Vaginal swabs (HVS) samples were directly spread on blood agar and MacConkey agar and incubated overnight at $37^{o}\pm1^{o}C$. The miscellaneous samples i.e. fluids, sputum; swabs etc. were processed as per the method described by Monica (1991).

Cultural and Biochemical Characterization of the Bacterial Strains

The bacterial isolates were characterized by inoculating on sheep blood agar and MacConkey agar. Randomly selected colonies were Gram stained, Oxidase test, Citrate utilization test, Methyl Red Voges Proskauer (MR-VP) Test, Nitrate reduction test, Triple Sugar Iron Test for lactose/glucose fermentation, Mannitol and Sucrose fermentation test, Hydrogen sulphide (H_2S) production, Indole production test, Urease test, Motility test haemolysis, catalase test, coagulase test and DNase test (Monica, 1991).

Evaluation of β-lactamase

Iodometric detection of β-lactamase

The β-lactamase were identified using iodometric method (Sykes, 1978) and confirmed by the acidometric method (Monica, 1991).

Detection of Extended Spectrum β-lactamases (ESBL)

The double disc method was used to detect the presence of extended spectrum β-lactamase (ESBL). A single, well defined colony of the test organism was emulsified in physiological saline (0.9 %) in a test tube. The turbidity of the test organism was matched against the 0.5 McFarland standards. The suspension of the test organism was spread on Mueller-Hinton agar and disc of co-amoxiclave (amoxicillin20 µg/clavulanic acid10 µg) was placed at the centre of Petri plate on agar surface. Discs of cefotaxime, ceftriaxone, ceftazidime and aztreonam (30 µg each) were arranged in a way that the distance between the central disc and surrounding discs was about 30 mm and incubated at $37^{o}C$ for 24 hours. After the overnight incubation, the zones around the 3^{rd} generation cephalosporin and azteronam discs were observed. If the inhibition zone around one or more, cephalosporin and aztreonam discs was extended on the side nearest to the co-amoxiclave disc, organisms have a ESBLs producer. When there was no increase zone of inhibition, the test was repeated by reducing the distance between the co-amoxiclave and cephalosporin and aztreonam discs to 20 mm or even less. Zones of inhibition were again observed on the next day. If there was no increase in inhibition of zones of 3^{rd} generation cephalosporin and aztreonam towards co-amoxiclave discs, they were considered as ESBLs non-producers (Jarlier *et al*., 1988).

RESULTS

Prevalence of various clinical isolates, β- lactamase and Extended spectrum β- lactamase strains from hospitalized and out patients (OPD) at Post graduate Medical Institute, Hayatabad Medical Complex (PGMI, HMC) Peshawar (Pakistan), was investigated during January 2000 to December 2003.

Prevalence of Bacterial Isolates during the period 2000-2003

During the period 2000 to 2003, 34.90% clinical samples showed bacterial growth. These organisms were isolated and identified. Among them 36.25% was Gram positive while 63.75% were Gram negative. The most prevalent organism was found to be *E. coli* (39. 45%), followed by *S. aureus* (32.23%), *Proteus spp.* (9,23%), *Pseudomonas spp*. (6.54%), *Streptococuss* (3.51%), *Citrobacter spp.* (2.80%), *Acinetobacter spp.* (2.66%), *Providencia spp.*

Table 1: Prevalence of Bacterial Isolates at Postgraduate Medical Institute, Hayatabad Medical Complex, Peshawar Pakistan in the period 2000-2003

Total Samples Received	2000		2001		2002		2003		Total	
	1678		2646		3829		4106		12259	
	n	%	n	%	n	%	n	%	n	%
Total samples growth	804	47.91	1085	41	1212	31.65	1178	28.69	4279	34.90
Total samples no growth	874	52.09	1561	59	2617	68.3	2928	71.31	7980	64.99
Gram positive	283	35.20	446	41.1	388	32.01	434	36.84	1551	36.25
Gram negative	521	64.80	639	58.89	824	67.99	744	63.16	2728	63.75
E. coli	327	40.67	392	36.13	511	42.16	458	38.88	1688	39.45
S. aureus	263	32.71	381	35.12	328	27.06	407	34.55	1379	32.23
Proteus spp.	84	10.45	82	7.56	138	11.39	91	7.72	395	9.23
Pseudomonas spp.	52	6.47	64	5.90	77	6.35	87	7.39	280	6.54
Streptococuss spp.	20	2.49	56	5.16	47	3.88	27	2.29	150	3.51
Acinetobacter spp.	7	0.87	48	4.42	40	3.30	19	1.61	114	2.66
Citrobacter spp.	41	5.10	28	2.81	19	1.57	32	2.72	120	2.80
Providencia spp.	10	1.24	12	1.11	16	1.32	56	4.75	94	2.2
Miscellaneous	0	0	22	2.03	36	2.97	1	0.08	59	1.38

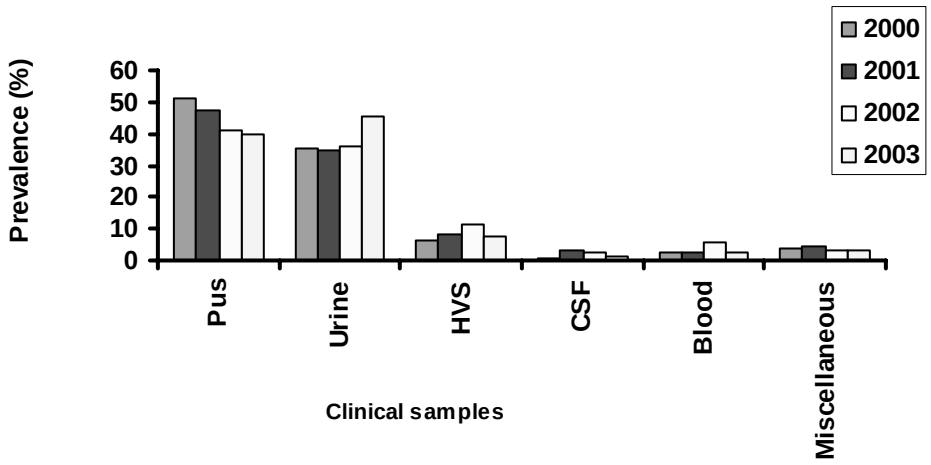

Fig. 1: Prevalence of clinical samples showing the growth of bacteria in Post graduate Medical Institute Hayatabad Medical Complex, Peshawar during the period 2000-2003.

(2.2%) and miscellanous (1.38%).Year wise results are shown in table 1.

Prevalence of clinical samples during the years 2000-2003

Various clinical samples like, pus, urine, HVS, CSF, blood etc were collected to study the prevalence of microorganism. Microorganisms in pus samples during the year 2000 to 2002 were abundant; however, urine samples showed the highest count during the year 2003. Generally, pus samples showed the highest count followed by urine samples (fig. 1).

Prevalence of bacterial isolates in different clinical samples during the years 2000-2003

Pus sample

S. aureus was found to be the most prevalent organism in pus samples during 2000-2003 study periods. The highest incidence was observed in the year 2001 but was not significantly different from other study period. The *E. coli* was the second most prevalent organism in pus samples. Fig. 2 indicates that *S. aureus* and *E. coli* are the major organisms found in pus samples.

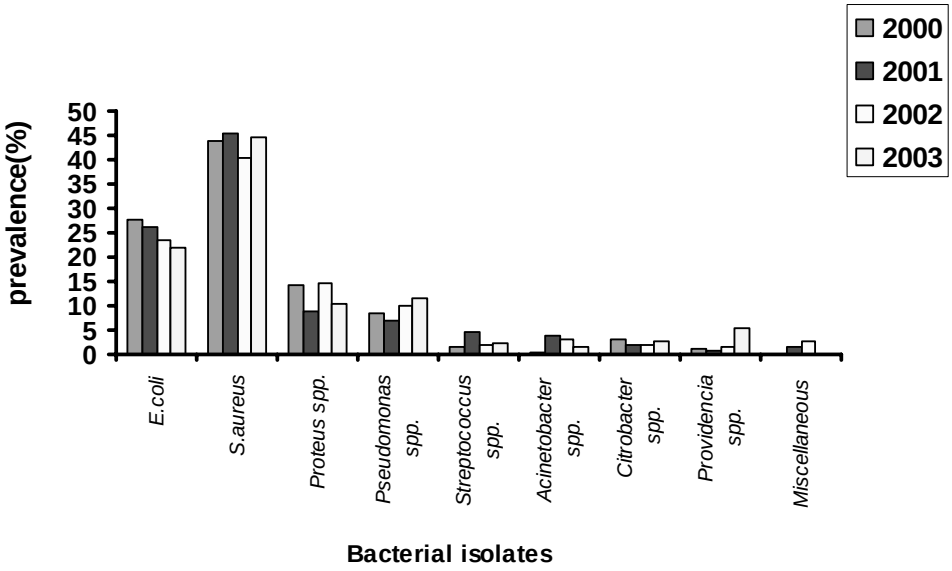

Fig. 2: Prevalence of Bacterial isolates in pus samples in Post-graduate Medical Institute, Hayatabad Medical Complex, Peshawar during the years 2000-2003.

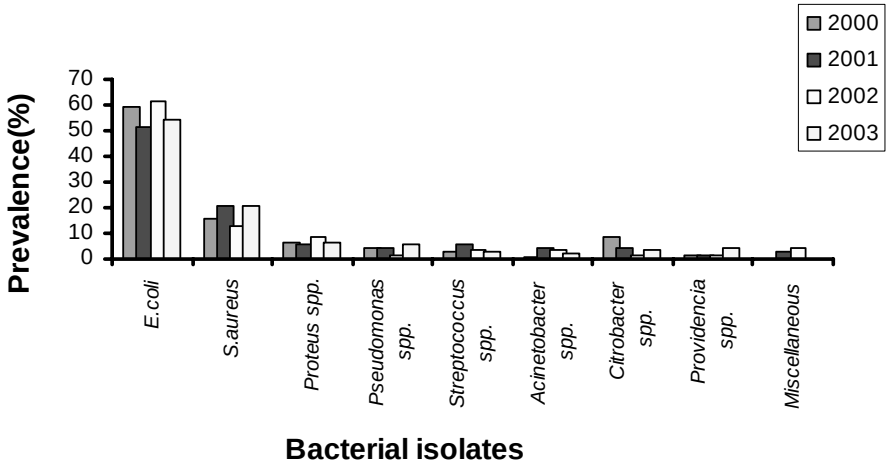

Fig. 3: Prevalence of bacterial isolates in urine samples in Postgraduate Medical Institute Hayatabad Medical Complex Peshawar during the years 2000-2003.

Urine samples

E. coli was found to be the most prevalent organism in urine samples. The highest prevalence was observed during the year 2002. *S. aureus* was the second most prevalent organism in urine samples. Fig. 3 shows the growth of various bacteria in urine samples.

High vaginal swab (HVS) samples

In HVS samples the *E. coli* was found to be the most prevalent organism. The highest prevalence was observed in the year 2000 but was not significantly different from the year 2001 to 2003. The *S. aureus, Streptococcus* and *Proteus spp.* were the most prevalent organism in HVS samples. The results are shown in fig. 4.

Cerebrospinal fluid (CSF) samples

E. coli, *S. aurus*, *Pseudomonas spp* and *Streptococcus spp.* were the most prevalent organism in CSF during the period 2000 to 2003.

Table 2: Month-wise prevalence of bacterial isolates during the year 2000-2003

Organisms	Total	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
E. coli	1688	115	79	110	153	127	155	146	160	192	198	132	121
S. aureus	1379	85	89	153	146	121	129	119	98	155	119	85	80
Proteus	395	31	33	37	39	28	36	25	20	29	44	37	36
Pseudomonas	280	20	22	46	25	18	28	13	20	17	34	20	17
Streptococcus	150	9	9	15	10	16	19	4	16	12	25	9	6
Acinetobacter	114	21	5	7	12	2	7	3	9	13	8	12	15
Citrobacter	120	17	7	18	10	13	13	9	8	5	3	9	8
Providencia	94	10	2	0	9	9	12	8	6	7	11	11	9
Misc.	59	3	6	3	7	14	9	4	4	9	1	0	1
Total	4279	311	252	389	411	348	408	331	341	437	443	315	293

Blood sample

S. aureus and *E. coli* were found to be the most prevalent organism in blood samples during the study. Prevalence of *E. coli* (55% and 61.97%) was the highest during the period 2000 and 2002, while the highest prevalence of *S. aureus* (46.15% and 75%) was observed during the period 2001 and 2003. The prevalence of other organisms was not significant during the study.

Miscellaneous samples

Apart from the major category of samples, urine catheter, stool, sputum, tracheal secretion, tissue and different types of swabs were also collected. In miscellaneous samples, *S. aureus* was found to the most prevalent organism and it prevalence was found to be 36.36%, 38.78%, 33.33% and 54.05% during the period 2000-2003, respectively. It was followed by *E. coli* and *Proteus spp.*

Gender-wise prevalence of bacterial isolates in Post graduate Medical Institute Hayadabad Medical Complex Peshawar during the years 2000-2003

The growth of microorganism was high in samples obtained in female patients compared with the samples obtained from male patients. Fig. 5 shows the significantly high rate of infection among female patients compared with the male patients. *E. coli* and *S. aurus* causes maximum infection in female as compared to male. Prevalence of *Proteus spp*. was not significantly different among the male and female patients, while *Pseudomonas spp.* cause infection maximum in male as compare to female. Infection due to *Streptococcus*, *Acinetobacter* and *Citrobacter spp.* are not significantly different in both genders. *Providencia spp.* infected the male more as compared to female. In miscellaneous isolates obtained from different sample, the infection in both sex were same.

Month-wise prevalence of bacterial isolates in Post graduate Medical Institute Hayatabad Medical Complex, Peshawar Pakistan during the years 2000-2003

Table 2 summarizes the month-wise prevalence of bacterial isolates during the period 2000 to 2003. *E. coli* was found to be the most prevalent in months of April to October and maximum infections were observed in month of September and October. Infections due to *S. aureus* were found maximum in the month of March to September and were most prevalent in the month of March, April and September. *Proteus spp.* was observed through out study period. *Pseudomonas* was prevalent maximum in the month of March while *Streptococcus, Acinetobacter, Citrobacter*, and *Providencia spp* were found same in all month during the study.

Evaluation of β-lactamase

The clinical isolates of *E. coli* and *S. aureus* were randomly collected, 75 samples were selected each in year 2001-2003 and 65 samples were selected in year 2000 for the β-lactamases and Extended spectrum β-lactamase studies.

The prevalence of β-lactamase producing *S. aureus* was studied using an iodometric method and further verified by rapid acidometric method. The insignificance increase (80% to 82.67%) were observed in β-lactamase producing *S. aureus* during the year 2000 to 2003, as shown in table 3.

In *E. coli* rapid increase in β-lactamase producer strains was observed. In year 2000, where 76.92% *E. coli* were found to be β-lactamase producers this figure increased to 81.33%, 82.66% and 85.33% from years 2001 to 2003, respectively. The results are shown in table 3.

Table 3: Prevalence of β-lactamase (BL) and ESBLs producers among clinical isolates of *E. coli* and *S. aureus* at Postgraduate Medical Institute Hayatabad Medical Complex, Peshawar, Pakistan, during the period 2000-2003.

Year	n	BL producer *E. coli* n (%)	ESBLs producer *E. coli* n (%)	BL producer *S. aureus* n (%)
2000	65	50 (76.92)	9 (13.85)	52(80%)
2001	75	61 (81.33)	11 (14.66)	61(81.33%)
2002	75	62 (82.66)	14 (18.67)	61(81.33%)
2003	75	64 (85.33)	17 (22.66)	62(82.67%)

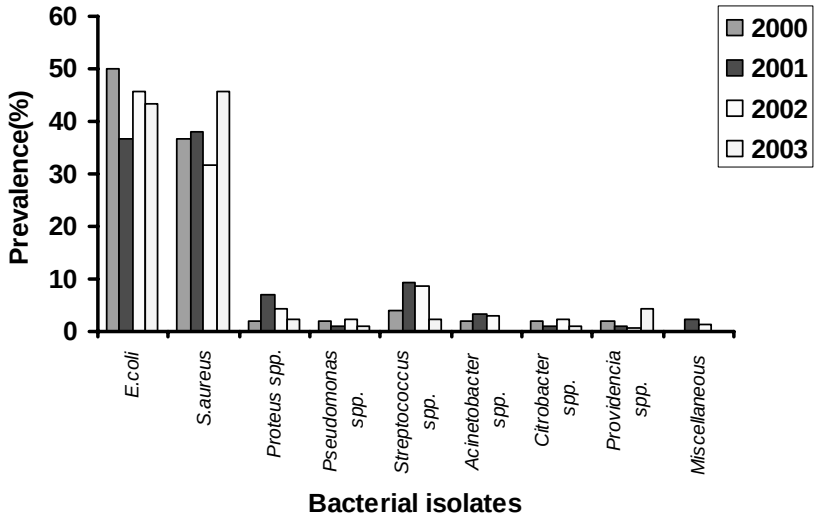

Fig. 4: Prevalence of bacterial isolates in high vaginal swab samples in Post-graduate Medical Institute Hayatabad Medical Complex, Peshawar during the year 2000-2003.

The prevalence of ESBL producer *E. coli* was studied using the double disc diffusion method. In year 2000, 13.85% *E. coli* were found to be ESBL producers and this figure increased to 22.66% in the year 2003. Maximum increase (4.01%) in ESBL producer *E. coli* was observed in 2002. The results are shown in table 3.

DISCUSSION

Prevalence of bacterial isolates during the year 2000-2003

During this study only 34.90% showed the bacterial growth. It has been observed that number of samples increased every year that may be due to either increase in population of N.W.F.P. or influx of the Afghan refuges that enter Pakistan via Peshawar border, specially in the year 2002 where 3829 clinical samples received compared with the year 2001 (n = 2646). The *E. coli* was found to be the most prevalent organism through out the study and its prevalence was 36.13% to 42.16%. The analysis of data using ANOVA test showed that year-wise differences in the percentage of *E. coli* are not significant different ($P > 0.05$). However, the increase was steady throughout the study. Maximum *E. coli* mediated infections (42.16%) were observed during the year 2002, followed by year 2003 (38.88%).

S. aureus was the second most prevalent organism ranging between 27.06% to 35.12%, maximum growth of *S. aureus* 34.55% observed in the year 2003. In contrast to *E. coli* minimum 27.06% *S. aureus* was observed in the year 2002. A negative correlation between the prevalence of *E. coli* and *S. aureus* has been observed. Prevalence pattern of *E. coli* and *S. aureus* in this study is also substantiated by other study (Gakuu, 1997), where *E. coli* and *S. aureus* were reported as the most frequently isolated organism in hospital.

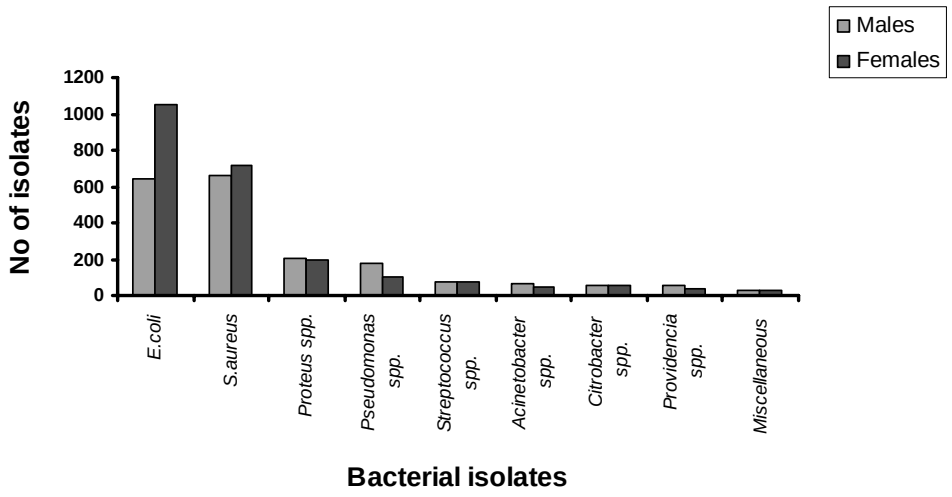

Fig. 5: Gender wise distribution of pathogenic bacterial isolates in Postgraduate Medical Institute Hayatabad Medical Complex Peshawar studied during 4 years i.e. 2000-2003.

The other most prevalent microorganisms were *Proteus spp.*, *Pseudomonas spp.*, *Streptococuss spp.*, *Citrobacter spp.*, *Acinetobacter spp*. and *Providencia spp.* The similar prevalence of bacteria was also observed by antimicrobial surveillance program in Europe (Fluit *et al.,* 2000).

Prevalence of Gram positive and Gram negative isolates in the year 2000-2003

The growth of the Gram negative organism was very high (63.75%) compared with Gram positive organism (36.25%). The most prevalent organisms among Gram positive and Gram negative organisms were *S. aureus* and *E. coli*, respectively. This study showed that the Gram negative being the most prevalent microorganism. These results are consistent with the similar studies in Europe (Fluit *et al.,* 2000).

Prevalence of biological sample

During the years 2000-2003, pus was found to be the most frequent biological sample, followed by urine, HVS, CSF, blood and miscellaneous samples. The high percentage of pus sample was observed from 2000 to 2002, while in the year 2003 the urine was observed to be the most prevalent sample, HVS was found maximum in the year 2002 , CSF was found maximum in the year 2001 and 2002, blood was found maximum in the year 2002. The high percentage of pus samples may be due to the various infections, particularly wounds. The use of appropriate and rational use of antibiotic may help to reduce such infections.

Prevalence of bacterial isolates in different biological samples

The evaluation of data of pus sample showed that *S. aureus* was the most prevalent organism during the year 2000 to 2003. Gram positive cocci are commonly found in pus, urine and blood samples (Pohlod *et al.,* 1987). The similar studies in Norwegian and Portuguese pediatric hospitals for three years showed the high prevalence of *S. aureus* from clinical specimens of pus, urine, and blood (Andersen *et al.,* 2000) and also from wound, blood, lungs, bone, joint and heart valves samples (Espersen, 1995 and Shi *et al.,* 1998). A high prevalence of *S. aureus*, in Islamabad, Pakistan has also been reported (Siddiqi *et al.,* 2002).

E. coli was found to be the second next prevalent organism in pus samples during year 2000-2003, followed by *Proteus spp.*, *Pseudomonas spp.*, *Streptococuss spp.*, *Acinetobacter spp.*, *Citrobacter spp.* and *Providencia spp*. These results are supported with other finding (Fluit *et al.,* 2000).

E. coli was the most common isolates in urine samples during the years 2000-2003. The linear increase of the *E. coli* isolates in urine samples was observed during the years 2001-2002. The *E. coli* has been found to be the main organism isolated from urine in the urinary tract infection (Orrett, 2003). This may be due to large number of female patients having attended the hospital as has been reported previously (Carrmen, 2005). During 2002-2003, the high number of female patients may be due to

migration of Afghan families. This may be the cause of high number of urine samples.

S. aureus was observed to be second most prevalent organism in urine samples, followed by *Proteus spp.*, *Citrobacter spp.*, *Pseudomonas spp.*, *Streptococuss spp.*, *Acinetobacter spp.*, *Providencia spp.* and miscellaneous. *E. coli*, *S. aureus* and *Proteus spp.* have been reported in other study during period 2000-2003 to be the most prevalent organism in urine (Muratani and Matsunoto, 2004).

E. coli was the most prevalent organism in HVS, CSF and blood samples, that was followed by *S. aureus* and *Streptococuss spp.*. These results are in line with the other finding (Shah *et al.,* 2002, Bema *et al.,* 2004). Studies in USA, Canada and Latin America also reveal that the *E. coli* has been the major organism for blood infections (Fluitt et al., 2000); our findings also showed the similar pattern of blood infections.

In the present study, *E. coli* and *S. aureus* have been identified as major bacterial pathogen contributing towards hospital infections, from the sample set obtained. *E. coli* has been recognized as an important potential human pathogen. *E. coli* forms part of the normal microbial flora of the human and animal intestinal tract, yet also found in water, soil and vegetation. It is not normally pathogenic to humans but may be referred to as an opportunistic pathogen, often associated with urinary tract infection (including cystitis, pyelitis and pyelonephritis) wound infection, appendicitis, peritonitis, gall bladder infection, bacteremia, neonatal meningitis, disease and sepsis (Mahon and Manuselis, 1995).

S. aureus is mainly responsible for localized and systemic infections. Despite the use of potent antibiotic, high mortality still exists after *S. aureus* isolates infections. Nosocomial multi drug resistant *S. aureus* is an important health care problem world wide (Espersen, 1995).

Gender-wise prevalence of bacterial isolates during the year 2000-2003

E. coli was the major clinical isolate obtained from females patients samples (62%) compared with the sample obtained from the male patients (38%). While in case of infection caused by *S. aureus*, the percentage of male and female patients was 47.9 and 52.1, respectively which is statistically not significant ($p > 0.05$). The gender-wise difference between the samples showed the growth of *Proteus spp* was not significantly different. However *Pseudomonas* isolates were significantly higher in samples obtained from males (63.2%) compared with those obtained from females (36.8%).

The growth of the *Streptococuss spp.* was insignificantly higher in females (52.67%) compared with males (47.33%). The similar results for growth of *Acinetobacter spp.* for male (57.02%) and female (42.98%) patients were observed.

The *Citrobacter* isolates were found in 120 samples that were obtained from equal number of male and female patients and in *Providencia spp.* male patients were 60.64% and female patients were 39.36%.

Khan *et. al*. (2002) reported more prevalence of *E. coli* in females than males, as 63.6% of urine samples were positive for *E. coli* from female patients. These finding are also in conformity with those reported by other researchers. The reason for high prevalence in case of females is that the females have open genitalia, predisposing it to faecal contamination, as compared to males whose relatively closed genitals prevent the establishment of pathogens. *E. coli* easily spreads to vaginal passage through faecal contamination, where invades and colonizes in the urinary tract leading to infection (Sotto *et al.,* 2001). Similar sex wise distribution pattern between male and female in stool samples was also reported by Khan *et al*. (2002). The women are often associated with various household activities, such as cleaning of toilets/bathrooms, food preparation and changing diapers of babies, as well as potty training their children may be the possible reason for high prevalence of bacterial infections. This inevitably leads to contamination of hands and nails which may result in the entry of the pathogen through the oral route (Chowdhary *et al.,* 1994). This may also explain the high incidence of U.T.I. among women as well. However, hands are washed more frequently and greater care is emphasized in this regards, the rate of infection through the faecal oral route is far less than the genito-urinary tract (Chowdhary *et al.,* 1994; Sotto *et al.,* 2001).

Urinary tract infection (U.T.I.) is the most common bacterial infection in women and it occurs with much greater frequency among elderly compared to younger women, with increasing frequency among post-menopausal women (Raz, 2001). *E. coli* is the main causative agent of uncomplicated U.T.I. and accounts for more than 85% of recurrent cystitis, and at least 32% of recurrent pyelonephritis (Barnett and Stephens, 1997). The reservoir for uropathogenic *E. coli* is faecal flora, from which bacteria spread to the urogenital mucosa, ascend to involve the ureter and kidney pyelonephritis (Langermann and Ballon, 2001).

Month-wise prevalence of bacteria isolates

E. coli was found to be the most prevalent organism during the months of April to October (except May and July) during the year 2000-2003. The occurrence of *E. coli* was observed to be maximum in the month of September and October. In these months the temperature in Peshawar is between 32-37°C, which is the optimum

for the growth of *E. coli*. These results are in good agreement with data reported in other studies where *E. coli* infections were more common at temperature 32-37°C in Nigeria (Nzeako *et al.,* 2002).

The growth of *S. aureus* isolates was common in samples collected during March to September (except July) during year 2000-2003. The occurrence of *S. aureus* was observed to be maximum in the month of September, March and April. In these months the temperature of Peshawar is between 30-37°C. These finding are agreement with those reported elsewhere (Chauhan *et al.,* 2004).

Pseudomonas was found maximum in the month of March. The *Proteus* growth and other organisms was scattered in samples collected throughout the year with a little variations.

β-lactamases

The linear increase in prevalence of β-lactamase producing *S. aureus* and *E. coli* was observed during the period 2000 to 2003. The increase in resistance rate may be due to the increase in the β-lactamase producing organism. The high percentage (80%) of *S. aureus* have been reported to be the β-lactamase producers in Karachi, Pakistan (Harris, 2001) and the similar results were also reported in other parts of the world (Chong, 1983; Shibli, 1992). The present data from Peshawar (N.W.F.P.), Pakistan also show the similar pattern.

Prevalence of ESBL in *E. coli* was observed with a linear increase. These findings are in agreement with the results of Aamir *et al.* (2003) who reported 28.57% of ESBL producer *E. coli* from clinical isolates. These findings are further supported by Motta *et. al*. (2003) who observed 15.4% *E. coli* as ESBL producers in Brazil.

ESBLs have been found in Enterobacteriaceae from patients through out the world; however, the true prevalence is unknown. Admission to a nursing home, excessive antibiotics exposure, extended hospital stay, recent surgery, admission to an ICU and instrumentation have been identified as risk factors for the selection of ESBL producing strains (Lucet *et al.,* 1996).

Clinicians will find this information useful in prescribing effective and inexpensive antimicrobial agents against infections. Such findings are indicative of the overall health care system within Pakistan and the condition that most of the hospitals lack in proper post operative care, patient awareness and hygiene value.

CONCLUSIONS

S. aureus and *E. coli* was found to the most prevalent organism responsible for nosocomial infections. High percentage of β- lactamases were observed in *S. aureus* and *E. coli* and is felt to establish the antibiotic prescribing surveillance program in various heath care systems. The present findings will provide information to clinicians in prescribing effective and appropriate antimicrobial agents against infections and will help improve health care system in general and hospitals in particular.

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