EMERGENCE OF MULTI DRUG RESISTANT BACTERIA IN SUPPURATIVE INFECTION: A THREAT FOR TOMORROW

Kothari S[1*], Patel P[2], Bharat S[3]

1. Dr. Saroj Kothari, M.D. Pharmacology (Corresponding author)

Associate Professor, Pharmacology, G. R. Medical College, Gwalior, M.P, India

2. Dr. Poonam Patel

Junior resident II, Pharmacology, G. R. Medical College, Gwalior, M.P., India

3. Dr. Savita Bharat M.D. Microbiology

Associate Professor, Microbiology, G. R. Medical College, Gwalior, M.P, India

Summary

This study is planned to find out bacteria and their antimicrobial sensitivity from pus specimens collected in tertiary care teaching hospital.

Pus specimens were collected from July 2010 to December 2010 from suspected patients reported in outdoor and indoor departments of G.R. Medical College and J.A.Group of Hospitals Gwalior. The bacteria were cultured on Mac Conkey's agar and Nutrient agar, followed by the identification of the isolates based on their cultural characteristics and their reactions in standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique on Muller Hinton Agar

600 isolates were obtained from 835 pus swabs, of which 66.16% were from indoor samples. Isolated organisms include gram positive bacteria: *Staphylococcus aureus 48.83%,* gram negative bacteria*: Klebsiella 31.83%,Escherichia coli 9.83%, Pseudomonas aeruginosa 6.66%, Proteus 1.66%*, *Acenatobacter 0.66%* and *Citrobacter* species 0.5%.*Staphylococcus aureus* were sensitive to cephalosporins, vancomycin, quinolones, amoxiciilin - clavulinic acid, aminoglycosides and doxycycline but were resistant to ampicillin, amoxicillin, erythromycin and cotrimoxazole. 70% of the *Staphylococcus aureus* isolates were MRSA. All gram- negative bacteria were highly sensitive to cefoperazone except *Klebsiella species. Eshcherichia coli,Pseudomonas aeruginosa and Citrobacter* species were also sensitive to aminoglycosides and quinolones. All the gram negative bacteriawere resistant to ampicillin, amoxicillin,ticarcillin, erythromycin, tobramycin, cotrimoxazole, cefixime and cefuroxime.

Regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. The knowledge of the susceptibility patterns of the bacterial strains in a hospital will guide the clinicians to choose appropriate antibiotics for surgical prophylaxis and treatment.

.

Key Words: Pus, antimicrobial susceptibility, resistant microorganisms

Introduction

Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness and greater risk of death. When infections become resistant to first-line medicines, more expensive therapies must be used. The longer duration of illness and treatment, often in hospitals, increases health-care costs and the financial burden to families and societies[1].Irrational and inappropriate use of antimicrobials medicines provides favorable conditions for resistant microorganisms to emerge, spread and persist and is by far the biggest driver of drug resistance worldwide[2]. Drug resistance threatens to erase gains made in disease treatment and control in developing countries. No action today means no cure tomorrow[3].Incidence of pus producing infection is increasing[4].To establish any gains in control measures of such infections, it is very crucial for every institution to determine the specific pattern of pus producing microbial colonization, and the antimicrobial sensitivity profile, so as to generate data that would help clinicians to choose the correct empirical treatment. Therefore, objective of this study was to determine bacteriological profile of pus producing infection and to know the antimicrobial susceptibility pattern of the isolates from pus specimens collected from indoor and outdoor patients being treated in tertiary care teaching hospital.

Materials and Methods

A retrospectivesurvey based study was designed to determine the distribution of bacterial pathogens and their susceptibility pattern from suspected cases of pus producing infections. Pus swabs collected for bacteriology and antimicrobial susceptibility from indoor and outdoor patients of Surgery, Orthopedics, Medicine, Pediatrics, Obstetrics and Gynecology, Ophthalmology and ENT departments of Gajara Raja Medical College and J.A.Group of Hospitals from July 2010 to December 2010 were analyzed. The categories of infections involved in the study included burns, bruises, bedsores, trauma wounds, post operation sepsis, cellulitis, ulcers, abscesses and osteomyelitis.

The bacteria were cultured on Mac Conkey's agar and Nutrient agar, followed by the identification of the isolates based on their cultural characteristics and their reactions in standard biochemical tests. All the isolates were tested for antimicrobial susceptibility by the disk diffusion technique on Muller Hinton Agar by Paper disks impregnated with antibiotics (Span diagnostics limited, Surat, India): Penicillins: Ampicillin (10mcg), Amoxicillin (10mcg), Oxacillin (1mcg), Ticarcillin (75mcg),); Amoxicillin-Clavulanic acid (20/10 mcg); Cephalosporins: Cefuroxime (30mcg), Ceftriaxone (30mcg), Cefoperazone (75 mcg), Cefixime(30 mcg);); Aminoglycosides: Gentamicin (10 mcg), Tobramycin (10 mcg), Amikacin (30 mcg), Quinolones: Ciprofloxacin (5mcg), Ofloxacin (5mcg), Gatifloxacin (5mcg); Tetracyclines : Doxycycline (30mcg),); Macrolides: Erythromycin (15mcg) and Miscellaneous: Chloramphenicol (30 mcg), Cotrimoxazole (25mcg) respectively. A pre-diffusion time of 30 min was allowed at room temperature and the plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured and compared to that of standard strain and the results were

interpreted as sensitive, or resistant, based on Clinical Laboratory Standard Institute 2007 guidelines.[5] The percent antimicrobial susceptibility of the isolated microorganism against different antimicrobials tested were calculated and interpreted as highly sensitive, moderately sensitive, less sensitive and resistant for 90-100%, 80-89.99%, 60-79.99% and 0-59.99% susceptibility respectively.

Results

A total number of 600 isolates were obtained from 835 pus swabs collected from indoor and outdoor patients. 28.75% samples showed no growth after 24 hours. Solitary isolates were cultured from as many samples whereas twin isolates were cultured from only 5 samples (Table1).

Table 1: Number of bacterial isolates cultured from pus specimens

Isolates	**Number**
Solitary	600
Twin	5
No growth	230
Total	835

Among the 600 microorganisms isolated and studied 66.16% were from indoor and 33.84% were from outdoor samples. About the prevalence of isolates from indoor samples 86.64% were from surgery whereas only 5.79% were from orthopedics and 7.55% were from Medicine, Paediatrics, Obstetrics and Gynecology, Ophthalmology and ENT wards [Table 2].

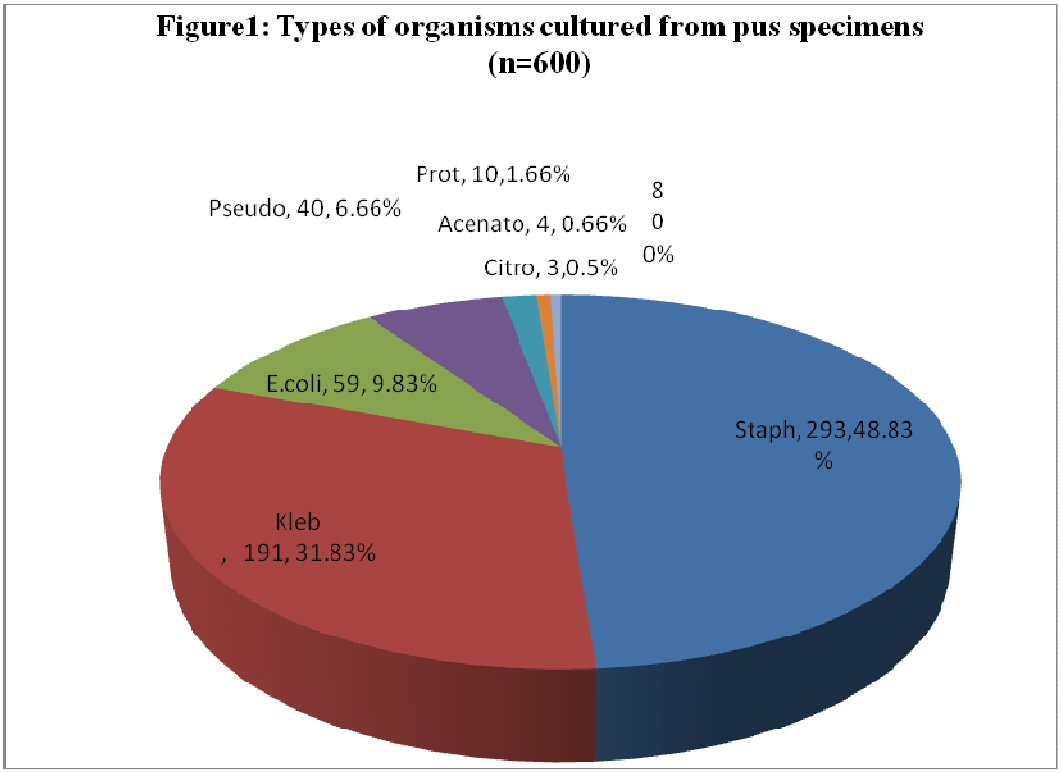
Table 2: Distribution of bacterial isolates cultured

Organisms	OPD	Surg	Ortho	Med	Paed	O&G	Oph	ENT	Total
Staph	137	133	11	9	3	2	1	-	293
Kleb	41	129	9	3	3	4	1	1	191
E.coli	11	46	-	2	-	-	-	-	59
Pseudo	10	23	3	2	-	2	-	-	40
Prot	3	7	-	-	-		-	-	10
Acenato	-	4	-	-	-	-	-	-	4
Citro	1	2	-	-	-	-	-	-	3
Total	203	344	23	16	3	8	2	1	600

Staph=Staphylococcus aureus, Kleb=Klebsiella species, E.coli=Eshcherichia coli, Pseudo=Pseudomonas aeruginosa, Prot= Proteus species, Acenato= Acenatobacter species, Citro=Citrobacter species, --=absent, OPD=outpatient department, Surg=surgery, Ortho=orthopaedics, Med=medicine, Paed=paediatrics, O&G=obstetrics and gynecology, Oph=ophthalmology, ENT= Ear Nose and Throat

Among the organisms isolated, 48.83% were *Staphylococcus aureus,* 31.83 % were *Klebsiella,* 9.83% were *Escherichia coli*, 6.66% were *Pseudomonas aeruginosa*, 1.66% were*Proteus*, 0.66% were *Acenatobacter* and 0.5% were *Citrobacter* species [Figure1].

Figure1: Types of organisms cultured from pus specimens (n=600)



Staphylococcus aureus were highly sensitive to cefoperazone, moderately sensitive to gatifloxacin, vancomycin, doxycycline, gentamycin, amoxiciilin – clavulanic acid, ofloxacin, less sensitive to amikacin, ceftriaxone, ciprofloxacin and chloromphenicol whereas resistant to amoxicillin (75.97%), cefixime (72.45%), erythromycin (70%), cloxacillin (70%), cotrimoxazole (57.41%), ampicillin (55.86%) and cefuroxime (42.40%) [Table 3]. *Klebsiella* did not show high or moderate susceptibility to any of the antimicrobial tested and were less sensitive to cefoperazone, gatifloxacin, amikacin, vancomycin, ofloxacin and ceftriaxone. *Klebsiella* were resistant to cefuroxime (93.75%), ampicillin (90%), cefixime (88.58%), cotrimoxazole (86%), erythromycin (85.19%), amoxicillin (83.34%) and ticarcillin (80%). *Eshcherichiacoli* were highly sensitive to cefoperazone, gatifloxacin and amikacin, moderately sensitive to gentamycin and vancomycin, less sensitive to doxycycline, ofloxacin, ciprofloxacin, cefuroxime, chloramphenicol and were resistant to cefixime (83.36%), ampicillin (83.36%), tobramycin (75%), ticarcillin (67%), amoxicillin (66.66%) and ceftriaxone (62.50%) [Table3].

Table3: Antibiotic susceptibility pattern (%) of pus isolates against different group of antimicrobials

Antimicrobials	*Staph*	*Kleb*	*E.Coli*	*Pseudo*	*Prot*	*Acenato*	*Citro*
Cefoperazone	97.22	78.57	100	100	100	100	100
Gatifloxacin	86.20	77.77	94.73	68.18	0	0	100
Amikacin	77.88	70.40	93.33	87.50	50	50	100
Vancomycin	85.78	69.23	80	60	--	0	0
Gentamycin	81.28	34.14	84.37	76.79	--	--	100
Amoxicillin-Clavulanic acid	80.50	14.28	60	41.66	0	0	100
Doxycycline	84.08	46.83	78.75	45	50	50	-
Ofloxacin	80.28	66	68.75	40	0	20	-
Ceftrioxone	71.42	31.48	37.50	62.50	45	33	0
Ciprofloxacin	61.60	44	64	57.14	33	--	50
Chloromphenicol	60	41	60	0	66	--	--
Cotrimoxazole	42.59	14	52.94	22.22	--	--	--
Ampicillin	44.14	10	16.66	16.16	0	--	--
Amoxicillin	24.03	16.66	33.33	9.09	0	--	0
Cefuroxime	57.60	6.25	60	8.33	25	33	50
Erythromycin	30	14.81	--	--	--	--	-
Cefixime	27.55	11.42	16.66	27.27	--	--	100
Cloxacillin	30	--	57	--	--	--	--
Ticarcillin	58	20	33	28	28	0	0
Tobramycin	50	33	25	20	25	0	0

Staph = Staphylococcus aureus, Kleb=Klebsiella species, E.coli=Eshcherichia coli, Pseudo=Pseudomonas aeruginosa, Prot= Proteus species, Acenato= Acenatobacter species, Citro= Citrobacter species,-- = not tested

Pseudomonas aeruginosa were highly sensitive to cefoperazone and ceftriaxone, moderately sensitive to amikacin, less sensitive to gentamycin, gatifloxacin, ceftriaxone and vancomycin whereas resistant to chloramphenicol (100%), cefuroxime (91.67%), amoxicillin (90.91%), ampicillin (83.84%), tobramycin (80%), cotrimoxazole (77.78%) and cefixime (72.73%). *Proteus* species were highly sensitive to cefoperazone, less sensitive to chloramphenicol and were resistant to amoxicillin-clavulanic acid, gatifloxacin, ampicillin, amoxicillin, ofloxacin (100%), tobramycin (75%), ticarcillin (72%) and ciprofloxacin (67%). *Acenatobacter* species were highly sensitive to cefoperazone and resistant to all other antimicrobials tested. *Citrobacter* species were highly sensitive to cefoperazone, amikacin, gentamycin, amoxicillin-clavulanic acid, gatifloxacin and resistant to ceftriaxone, vancomycin, cefixime, cefuroxime, ticarcillin, tobramycin (100%) and ciprofloxacin (50%) [Table 3

Discussion

Suppurative infections of the skin are common occurrences in hospitalized and out patients with lowered host resistance such as damaged skin and mucous membrane, where it may produce skin lesion such as boil or surgical site infections[6]. The results of this study show that *Staphylococcus aureus* is the leading etiological agent (48%) of pus producing infections. This is in agreement with the previous reports[7]. Among the isolated organisms 53.25% of the gram positive organisms and 75% of the gram negative organisms are from indoor samples indicating nosocomial infection is a major problem. Surveillance of nosocomial infections with an emphasis on antimicrobial audit will reduce the risk of post-operative infection[8].In this study *Staphylococci* were resistant to first line drugs. Data received from several years show an increasing resistance for drugs that were once considered as the first line of treatment for *Staphylococcal* infection[9].Present study show 70% of the *Staphylococcus aureus* isolates were MRSA inferred on the basis of their resistance to oxacillinwhich are in accordance with the earlier reports showing existence of 83% MRSA from pus samples received from different departments of the hospital and only a few patients from ICU[10]. Emergence of MRSA has not only caused therapeutic problems in hospitals but also put a tremendous pressure on resources for controlling their spread.

Vancomycin should only be used for the treatment of MRSA and in MSSA infections when patient is allergic to nafcillin, oxacillin and cloxacillin. 15% of the MRSA showed non susceptibility to vancomycin and 3% to the cefoperazone. Lack of control over use of these antimicrobials might have led to present resistance observed. These drugs should not be used as empirical therapy otherwise there are ample chances for development of resistant strains which would be resistant to almost all antibiotics[11]. Contact precautions recommended by the Centers for Disease Control and Prevention for hospitalized patients with MRSA include use of a private room, wearing gloves on entering the room, wearing a gown if contact with the patient or items in the room is anticipated, and hand washing on removal of the gloves[12].might be followed in order to fight against MRSA. The high sensitivity of the tested organism to gatifloxacin may not serve any useful purpose because its use is banned. Cefoperazone should also be used with caution in adults because the emerging low level of resistance may become high in future due to selective pressure of exposure as a result of constant use because of arbitrary prescription of the antibiotic.Teicoplanin, linezolid and teigecycline might be used as reserve drugs to treat vancomycin resistant *staphylococcus aureus*[13].

Klebsiella is regarded as one of the most frequent among gram negative organisms causing nosocomial infections producing pus. In the present study 62% of the gram negative organisms are *Klebsiella spp*. Majority of samples were received from surgical department alone.Surgical wards are the units where patients are hospitalized for a long period of time and medical equipments are used extensively. In the present study *Klebsiella* species were not 100% sensitive to any of the antimicrobial tested, suggesting inappropriate use of antibiotics has led to their decreased effectiveness[14].The overall resistance to various generations of cephalosporins was high which might be on account of this organism harbors series of antibiotic resistant genes which can be transferred horizontally to other bacterial species[15]. Among the gram negative bacteria, *Klebsiella* species is at the top of the chart of bacterial resistance next to *Acenatobacter* and *Proteus* species because of extensive prevalence in the hospital setting, patients easily become colonized and at an increased risk of developing infection with this organism and it

produces extended spectrum beta lactamases. In this study, 22% of the *Klebsiella* produced extended spectrum beta lactamase, which is inferred based on their resistance to cefoperazone and this finding is in accordance to previous study[16]. Owing to the increasing trend of resistance in this strain, the incidence of *Klebsiella* infection still carries significant mortality, in spite of the advances in sanitation facilities and introduction of wide varieties of antibiotics with anti *Klebsiella* activity. A new approach is required to overcome this nagging problem, where the *Klebsiella* infection might be treated preferentially based on epidemiological data and local susceptibility patterns considering the locally available antibiotics in the set up. Antimicrobial susceptibility of *Klebsiella* revealed high level resistance to common antibiotics encountered and is an indication that control measures have to be put in place, particularly in the administration of antibiotics in the hospitals. Patients should be educated on the consequences of indiscriminate use of antibiotics and there should also be an antibiotic resistance surveillance scheme. Carbapenem can be used as reserve drug against multi drug resistant *Klebsiella* species.

Eshcherichia coli are the third most common organisms isolated from pus. Emerging resistance (7-20%) to amikacin, gentamycin and vancomycin and high resistance to cefixime, ampicillin, tobramycin amoxicillin, ceftriaxone, cotrimoxazole might be due to permeability barrier, or changes in either outer membrane proteins and lipopolysaccharides of the isolated *Eshcherichia coli* as reported in earlier studies[17].Amikacin and cefoperazone seems to be promising for *Eshcherichia coli* infection in present study hence, their use should be restricted to severe nosocomial infections, in order to avoid rapid emergence of resistant strains.

Out of 40 culture positive *Pseudomonas aeruginosa* isolated in the present study, 60% were from surgical wards, indicating that nosocomial infection is the most common (Table 3). *Pseudomonas aeruginosa* were resistant to ampicillin, amoxicillin, antipseudomonal uriedopenicillin: ticarcillin (72-90%). This resistance may be due to production of type1 AmpC β-lactamase and low outer membrane permeability[18].*Pseudomonas aeruginosa* showed non susceptibility (72-90%) to oral cephalosporins. Our results are in corroboration with the one reported by other workers, showing high resistance to various generations of cephalosporins, on account of the production of extended spectrum β-lactamses (ESBLs) by the bacteria involved[19]. The resistance may also be due to the production of metallo-β-lactamases (MBL), which can be chromosomally encoded or plasmid mediated[20]. Emergence of resistance (20%) to amikacin against *Pseudomonas aeruginosa* in present study might be due to the production of enzymes that inactivates amikacin by phosphorylation, adenylation or acetylation[21].Resistance to chloramphenicol, doxycycline and less sensitivity to fluroquinolones against *Pseudomonas aeruginosa* in present study is in corroboration with earlier work reporting limited use of these classes of antibiotics[22].In order to prevent resistance to cefoperazone against *Pseudomonas aeruginosa* a therapeutic alternative as imipenem or merupenem has been recommended.

Today *Proteus* organisms are known to cause significant clinical infections and occupy multiple environmental habitats. Our results show that 70% of *Proteus* isolates from pus specimens were from surgical wards.This study clearly show *Proteus* resistance to most of antimicrobials used and it becomes necessary to have a continued surveillance of the organisms, to alleviate morbidity and mortality rates due to these organisms in Teaching Hospital. *Proteus* species usually show high resistance to commonly used antibiotics[23]. High resistance to multiple antimicrobials might be due to transfer of plasmid resistant genes among the gram negative organisms[24]. Third generation intravenous cephalosporins found highly effective in present study should not be used empirically so as to prevent resistance to this antimicrobial. Carbapenems can be used as reserve drugs.

Acenatobacter species cause significant clinical infections which are difficult to eradicate especially from hosts with complicated wounds, underlying diseases and the immuno-compromised[25].In the present study 4 *Acenatobacter species* were isolated and all were from surgical wards.Of the antimicrobial agents testedagainst *Acenatobacter species* only cefoperazone was effective. This is in accordance to earlier reports suggesting multidrug resistance among *Acenatobacter* isolates is commonly reported[26]. This finding also confirmed the fact that multidrug-resistant organisms may emerge more rapidly when the third-generation cephalosporins are used routinely, as has been predicted by other investigators[25].
Citrobacter species are aerobic, gram-negative bacilli commonly found in water, soil, food, and the intestinal tracts of animals and humans. *Citrobacter* bacteremia is uncommon and usually develops in patients with underlying diseases[27].Presence of *Citrobacter* in indoor and outdoor samples suggesting an increase in bacterial infection caused by *Citrobacter* species. Routine antimicrobial susceptibility revealed multidrug resistance to *Citrobacter* species and this may be attributed to the fact that these organisms are also harboring resistant genes as reported in earlier studies[27].

Conclusion

Control on infection due to multidrug resistance bacteria is the need of hour. Regular antimicrobial susceptibility surveillance is essential for area-wise monitoring of the resistance patterns. There is a need to introduce institutional antibiotic policy to restrict the occurrence of antibacterial resistance among the hospital strains and to prevent effectiveness of available antibiotics. We also advocate framing Drug and Therapeutic committee that will regulate appropriate and judicious use of these antibiotics.Screening for MRSA, VRSA and ESBL production need to be started as a routine procedure in clinical laboratoryto give valuable information to the clinicians to choose correct antibiotic for surgical prophylaxis and treatment. Updating the antibiogram periodically will further reduce the rate of nosocomial infections to a considerable extent.

Acknowledgments

The authors are thankful to Dr. (Mrs.) S. Gandhi Professor and Head and Miss Pooja Tomar Technical assistant Department of Microbiology, G. R. Medical College, Gwalior, (M.P.) for their unstinted help in collecting data. Authors express their sincere gratitude to Dr. (Mrs.) Shaila Spray, Dean, G. R. Medical College, Gwalior (M.P.) for her constant encouragement

References

1. The Bulletin of World Health Organization, Antimicrobial resistance 2010;88(11):797-876.
2. Rao GG. Risk factors for the spread of antibiotic resistant bacteria. Drugs 1998;55(3):323-30.
3. Jonathan DQ, Hans VH, German V &Lembit R. Bull World Health Organ Nov.2002;80(11):Genebra.
4. Mahmood K, Tahir T, Jameel T, Ziauddin A, Aslam HF. Incidence of Methicillin-resistant Staphylococcus Aureus (MRSA) Causing Nosocomial Infection in a Tertiary Care Hospital. Annals 2010;16(2): 91-96.
5. Clinical Laboratory Standards, C.L.S.I: Performance Standards for Antimicrobial Susceptibility Testing – Approved Standard. Clinical and Laboratory Standard Institute: Wayne PA – USA; 2008.
6. Collier M. Understanding wound inflammation. Nurs times 2003;99(25):63-64.
7. Shittu AO, Kolawole DO, Oyedepo EAR. Wound infections in two health institutions in IleIfe, Nigeria: Results of a cohort study. Osteomyelitis Wound manag 2003;49:52-57
8. Khorvash F, Mostafavizadeh K, Mobasherizadeh S, Behjati M, Naeini AE, Rostami S *et al.*, Antimicrobial susceptibility pattern of microorganisms involved in the pathogenesis of surgical site infection (SSI); A 1 year of surveillance, Pak J BiolSci I 2008;11(15):1940-4.
9. Ghosh A, Karmakar PS, Pal J, Chakraborty N, Debnath NB, Mukherjee JD. Bacterial incidence and antibiotic sensitivity pattern in moderate and severe infections in hospitalised patients. J Indian Med Assoc 2009;107(1):21-5.
10. Qureshi AH, Rafi S, Qureshi SM, Ali AM. The current susceptibility patterns of methicillin resistant Staphylococcus aureus to conventional anti Staphylococcus anti-microbials at Rawalpindi. Pak J Med Sci 2004;20:361-4.
11. Liu C, Chambers HF. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother 2003;47:3040–5.
12. Boyce JM, Havill NL, Kohan C, Dumigan DG, Ligi CE. Do infection control measures work for methicillin-resistant *Staphylococcus aureus*? Infect Control HospEpidemiol 2004;25:395–401.
13. Lentino JR, Narita M, Yu VL. New antimicrobial agents as therapy for resistant gram-positive cocci. Eur J ClinMicrobiol Infect Dis 2008;27:3-15.
14. Sekowska A, Janicka G, Klyszejko C, Wojda M, Wroblewski M and Szymankiewicz M. Resistance of *Klebsiella pneumonia* strains producing and not producing ESBL (extended spectrum beta lactamase) type enzymes to selected non-beta lactam antibiotics. Med SciMonit 2002;8(3):BR100-4.
15. Piddock LJV. Clinically relevant chromosomally encoded multi-drug resistance efflux pumps in bacteria. ClinMicrobiol Rev 2006*;*19(2):382-402.
16. Kusum M, Dhiraputra WS, Pongpech P and Naenna P. Occurrence of extendedspectrum beta-lactamase in clinical isolates of *Klebsiella pneumoniae*in a University Hospital, Thailand. J Med Assoc Thai 2004;87:1029- 33.

17. Cagnacci S, Gualco L, DebbiaE ,Schito GC and Marchese A, European emergence of ciprofloxacin resistant Eshcherichia coli clonal groups of 025:H4-ST 131 and015:K52:H1 causing community- Acquired Uncomplicated cystitis. J Clinical Microbiol 2008;46(8):2605-12.
18. Hancock RE. Resistance mechanisms in Pseudomonas aeruginosa and other non-fermentive gram negative bacteria. Clin Infect Dis1998;27:S93.
19. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum β-lactamase producing gram negative bacteria in a tertiary care hospital. Indian J Med Res 2002;115:153–7.
20. Navneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metallo β-lactamase producing Pseudomonas aeruginosa in hospitalized patients. Indian J Med Res 2002;116:264–7.
21. Poole K. Aminoglycosides resistance in Pseudomonas aeruginosa. Antimicrob Agents Chem 2005;49:479–87.
22. General Principles of antimicrobial therapy. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th ed. Mc-Graw Hill: Medical Publishing Division; 2006. pp. 1095–110.
23. Steward FS and Beswick TSL. Bactertiology, virology and immunity for students of medicine 10th Ed. ELBS and BalliereTindall London1977:235-237.
24. Yah SC, Eghafona NO, Oranusi S, Abouo AM .Widespread plasmid resistance genes among *Proteus* species in diabetic wounds patients in ABUTH Zaria. Afr J Biotechnol 2007; 6(15):1757-62.
25. Diane C.Halstead, Joan Abid, Micheal J. Diwzucky. Antimicrobial susceptibility among Acinetobactercal-coaceticus-baumannii complex and Enterobacbacteri-acae collected as part of the Tigecycline evaluation and surveillance trial. J Infect 2007;55:49-57.
26. Shi ZY, Liu PY, Lau Y, Lin Y, Hu BS, Shir J-M Antimicrobial susceptibility of clinical isolates of Acinetobacterbaumannii. DiagnMicrobiol Infect Dis1996 Feb;24(2):81-5.
27. Wang JT, Chang SC, Chen YC, Luh KT. Comparison of antimicrobial susceptibility of Citrobacter freundii isolates in two different time periods. J MicrobiolImmunol Infect 2000 Dec;33(4):258-62.