RESEARCH ARTICLE

A SOLUTION TO COMBAT AMINOGLYCOSIDE AND QUINOLONE RESISTANT GRAM NEGATIVE ORGANISMS

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ARTICLE INFO	ABSTRACT
	This study was carried out to know the prevalence of aminoglycoside and quinolone resistance among the collected isolates and to analyze the antibiotic susceptiblity patterns of various drugs against these isolates to find which drug offers the best solution against multidrug resistant Gram negative pathogens. Total 1824 clinical samples were collected from patients suspected of bacterial infection between March 2013 to May 2014. These samples were subjected for bacterial identification. The prevalence of aminoglycoside and fluoroquinolone among these isolates and antibiotic susceptibility testing were carried out according to the recommendations of Clinical Laboratory Standards Institute (CLSI) guidelines. Among the samples analyzed, 82.2 % (1499/1824) samples showed the growth of organisms of which 22 % (400/1824) were Gram positive and 60.2 % (1099/1824) were Gram negative and included in this study. Further analysis of Gram negative organisms revealed that 46.7% (513/1099) were aminoglycoside and 53.3% (586/1099) were quinolone resistant. Among Gram negative organisms that identified were *E. coli* (n=302)*, P. aeruginosa* (n=230)*, Acinetobacter species* (n=217) *K. pneumoniae* (n=150)*, Proteus species* (n=109) and *Citrobacter species* (n=91). Of the drugs tested, cefepime plus amkacin (Potentox) showed the highest activity against quinolone and aminoglycoside resistant Gram negative organisms with average susceptibility of >86% against all pathogens. Resistance to cefepime was 55%-74%, to tobramycin 20.8%-70.2%, to gentamycin 40%-65.6%, to levofloxacin 32.9%-62.4%, to moxifloxacin 40.1%-47.7%, to ofloxacin 29.9%-48.6%, to imipenem plus cilastatin 30%-60%, to ciprofloxacin 52.3%-75.7%, to ceftazidime 50.3%-60%, to azithromycin 40.5%-86%, and to amikacin 40.4%-79.7% among all isolated Gram negative bacterial pathogens. In conclusion, the results of this study strongly suggest the superiority of Potentox over other drugs and can be of effective alternative to treat infections caused by multi drug resistant Gram negative bacteria.

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INTRODUCTION

Fluoroquinolones (FQs) have been widely used to treat a number of infections, such as gonococcal, osteomyelitis, respiratory and urinary tract infections. They exert their activity by blocking DNA replication pathway (Yang *et al.,* 2010). High oral bioavailability, broad spectrum coverage and low toxicity make them excellent drugs for the treatment of these infections. However, over uses of FQs in the community is leading to resistance (Yang *et al.,* 2010; McDonald *et al.,* 2001) and thus affecting patient management (Solomkin *et al.,* 2010; Workwoski and Berman 2010). Resistance to FQs is

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rising rapidly, and in some parts of the world it has been reported to be >50% in *Enterobacteriaceae* (Dalhoff, 2012; Lautenbach *et al.,* 2001). Recently, it has been reported that use of levofloxacin is associated with increased resistance of P. aeruginosa to FQs (Lee *et al.,* 2010). Eighty-six percent of the ESBL-producing E. coli strains has been found to be resistant to levofloxacin in Shanghai, China (Xiong *et al.,* 2002). The increasing trend of FQs resistance in *A. baumannii* severely limits the usage of therapeutic antimicrobial agents (Chen *et al.,* 2011). Among the various mechanisms of resistance development, plasmid-mediated horizontal transfer of quinolone resistance genes *qnrA, qnrB* and *qnrS* play a vital role in FQs resistance development (Jacoby *et al.,* 2006; Robicsek *et al.,* 2006). These qnr genes have been widely reported in *Enterobacteriaceae, Shigella flexneri,* Citrobacter freundi**,** Providencia stuarti and Salmonella *species* (Tran *et al.,* 2005; Hopkins *et al.,* 2007; Wu *et al.,* 2007).

Aminoglycosides are also potent, broad spectrum antibiotics and have been used for the treatment of numerous life threatening Gram negative bacterial infections. They exert their antibacterial activity by inhibiting protein synthesis via binding to the 16S rRNA and by disrupting the bacterial cell membrane integrity (Shakil *et al.,* 2008). However, over the past few years, the emergence of resistant strains of *Pseudomonas species, E. coli, Klebsiella species, Acinetobacter species* has reduced the potential of aminoglycosides in empiric therapies (Gad *et al.,* 2011; Randhawa et al., 2004; Shahid and Malik, 2005**).** There are a number of aminoglycoside resistance mechanisms that include reduced uptake or decreased cell permeability (Wang *et al.,* 2003), alteration of the ribosomal binding site by rRNA methylases (Galimand e*t al.,* 2012; Doi and Arakawa, 2007), overexpression of efflux pump (Poole, 2004) and production of aminoglycoside-modifying enzymes (AMEs) (Kim *et al.,* 2009; Miró *et al.,* 2013). In Gram negative organisms, resistance to aminoglycosides such as amikacin, tobramycin and gentamycin has been reported to vary from 32.6% to 83.6% (Shahid and malik 2005).

In recent years, third, even forth generations of cephalosporin and other known effective broad-spectrum antibiotics are now being threatened by antibiotic resistance (Dua *et al.,* 2011). Besides extended spectrum beta-lactamases (ESBLs) resistance, resistance to quinolones and aminoglycosides is emerging fast and is difficult to recognize. Cefepime is commonly used in combination with an aminoglycosides for empirical therapy in the intensive care unit (ICU) for Gram-negative organisms particularly *P. aeruginosa* infections in critical care settings (Kollef 2003). In view of increasing incidence of antibiotic resistance to Gram-negative pathogens and failure of monotherapy, a combination therapy may be the only notable therapeutic approach to treat these infections caused by Gram negative antibiotic resistant organisms (Hughes *et al.,* 1997). The combination of aminoglycosides with beta-lactams has been documented to be synergistic (Sanz *et al.,* 2002). Increasing resistance to FQs and aminoglycosides encouraged us to study various drugs in order to find possible solution which could control resistance. The present study was conducted to find the prevalence of aminoglycoside and fluoroquinolone resistance among Gram negative pathogens and to evaluate the antibiotic susceptibility patterns of various drugs against these pathogens.

MATERIALS AND METHODS

Sample collection

A total of 1824 clinical samples of blood, sputum, urine, catheter tips and body fluids were collected from various hospitals between March 2013 to May 2014. The collection and processing of the samples were done according to a common standard operating procedure (SOP).

Isolation and identification of pathogens

All the samples were collected aseptically in sterile containers. Urine samples collected in sterile universal container and were directly inoculated onto the cystine lactose electrolyte deficient (CLED) medium.

Other specimens involving body fluids, sputum and catheter tips collected in sufficient amount and were inoculated on the different non-selective and selective culture media as per the standard microbiological techniques. Blood samples collected in brain heart infusion (BHI) broth in a ratio of 1:5 (blood/broth) were first incubated overnight at 37°C and then subcultured on to the non-selective and selective culture media. The organisms were identified on the basis of colony morphology, Gram staining, motility, and biochemical reactions (Cheesbrough, 2000; Nayar *et al.,* 2014; Bahashwan and Shafey, 2013).

Aminoglycoside and fluoroquinolone resistance detection

Fluoroquinolone resistant isolates were identified using norfloxacin 10 µg disc according to CLSI (2013). Aminoglycoside resistance was detected following the method described by Murdoch *et al.* (2003). A zone size of 6 mm in diameter indicated high-level resistance, and 10 mm indicated susceptibility. Zone diameters of 7 to 10 mm in diameter were considered intermediate.

Drugs

Antimicrobial susceptibility testing was done by Kirby–Bauer disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines (2013). The discs of following drugs gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), levofloxacin (5 µg), ofloxacin (5 µg), moxifloxacin (5 µg), ciprofloxacin (5 µg), imipenem plus cilastatin (10 µg), ceftazidime (30 µg), cefepime (10 µg), azithromycin (15 µg) and fixed dose combination (FDC) of cefepime + amikacin (Potentox) (40 µg) were procured from Himedia (Mumbai, India) and used in the study. Inoculum of 0.5 McFarland standards turbidity was prepared in a Mueller-Hinton broth (MHB, Hi-Media, Mumbai, India) from isolated colony of pathogens selected from 18–24 hour agar plates. Within 15 minutes, a sterile cotton swab was dipped into the inoculum suspension. The swab was rotated several times and pressed firmly against the inside wall of the tube above the fluid level and inoculated on the dried surface of a Mueller-Hinton agar (MHA) plate by streaking the swab over it. For even distribution of inoculum, the swab was streaked two more times at 60° over the agar surface. After 3–5 minutes, antibiotic discs were applied and pressed down to ensure complete contact with agar surface. The discs were distributed evenly to ensure a minimum distance of 24 mm from center to center. The plates are then inverted and incubated for 16-18 hrs aerobically at 37° C within 15 minutes of disc application. Sensitivity of isolated organisms against antibiotics was reported as sensitive (S) or resistant (R) based on the breakpoints.

RESULTS AND DISCUSSION

A total of 1824 clinical sample were collected from urine, blood, body fluids, catheter tips and sputum and processed for isolation of pathogenic bacteria. Out of the total samples analyzed, 1099 samples showed the growth of Gram negative organisms and 400 samples were of Gram positive pathogens (not included in this study) while 325 samples showed no growth of organisms.

Further analysis of Gram negative pathogens revealed that 46.7 % (513/1099) and 53.3 % (586/1099) pathogens were aminoglycoside and fluoroquinolone resistant, respectively (Table 1). Among the Gram negative samples showing growth, around 35.0 % samples were of urine followed by blood (23.6%), body fluids (19.0 %) and catheter tips (13.6%) and sputum (8.6 %) (Table 2).

As shown in Table 3, *E. coli* (27.5%) was found to be the most dominant pathogens which was comparable to previous study of Rajan and Prabhavathy (2012) where 34.8% prevalence of *E. coli* was reported. In another study, Shafiyabi *et al*. (2014) have also reported the prevalence of *E. coli* to be 39.6%. In our study, *Acinetobacter species*, *P. aeruginosa*, *K. pneumoniae*, *Proteus species* and *Citrobacter species* contributed 19.7%,

**Table 1. A profile of pathogens isolated from specimens**

Clinical samples	Total clinical samples	Samples not showing growth	Samples showing growth of Gram positive organisms	Samples showing growth of Gram negative organisms (%)	Prevalence of aminoglycoside resistance among Gram negative organisms	Prevalence of fluoroquinolone resistance among Gram positive organisms
Urine	595	105	105	385 (35.0)	170	215
Blood	478	98	120	260 (23.6)	128	132
Body fluids	295	41	45	209 (19)	96	113
Catheter tips	273	26	97	150 (13.6)	83	67
Sputum	183	55	33	95 (8.6)	36	59
Total	1824	325	400	1099	513	586

Table 2. Prevalence of Gram negative clinical isolates in samples showing aminoglycoside and quinolone resistance

Clinical samples	Samples showing growth of Gram negative pathogens	*Acinetobacter species*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*Citrobacter species*	*Proteus species*
Urine	385	78	98	83	66	27	33
Blood	260	58	79	55	28	18	22
Body fluids	209	33	64	41	21	20	30
Catheter tips	150	29	35	31	25	17	13
Sputum	95	19	26	20	10	9	11
Total (%)	1099	217 (19.7)	302 (27.5)	230 (20.9)	150 (13.6)	91 (8.3)	109 (10)
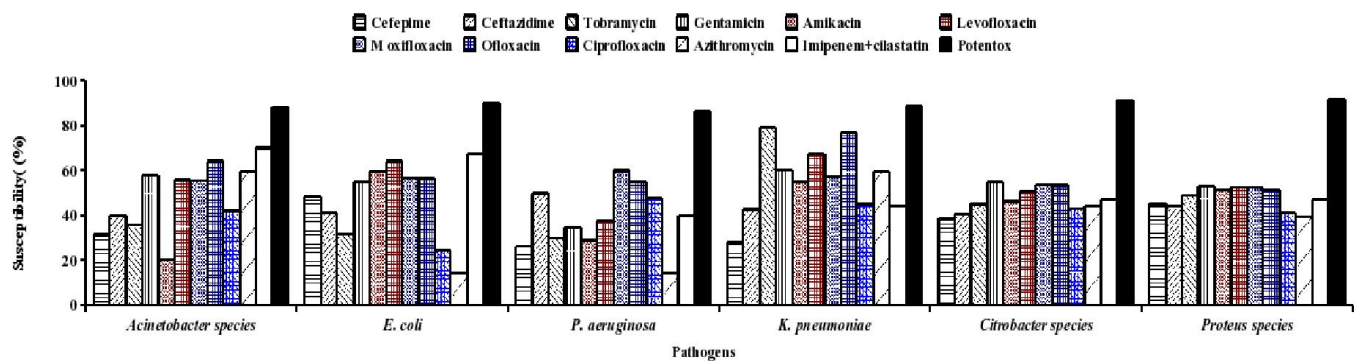
Figure 1. Antibiogram showing percentage susceptibility against aminoglycoside and fluoroquinolone resistant Gram negative pathogens
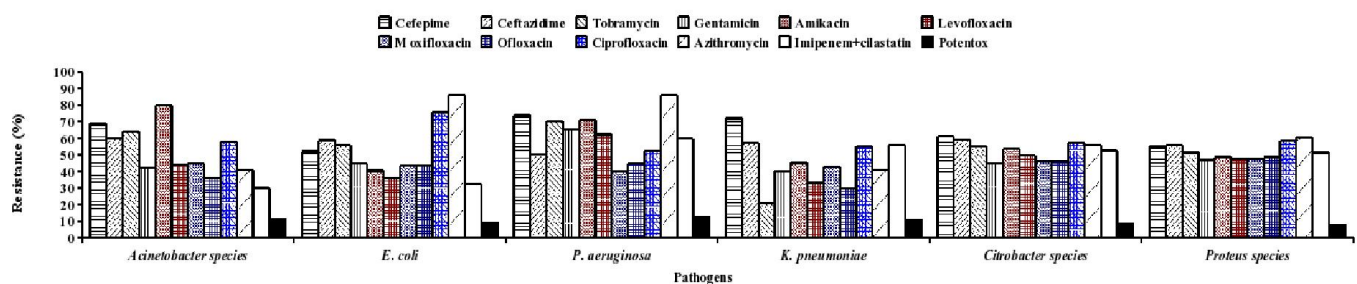
Figure 2. Antibiogram showing percentage resistance against aminoglycoside and fluoroquinolone resistant Gram negative pathogens

Morphological and biochemical characterization of the Gram negative samples (n=1099) revealed presence of 6 different Gram-negative organisms that includes *Acinetobacter species*, *E. coli, P. aeruginosa, K. pneumoniae, Citrobacter species* and *Proteus species*. The detailed profile of various organisms collected from various clinical samples is shown in Table 2.

20.9%, 13.6%, 10% and 8.3%, respectively to the isolated pool of pathogens. Bahadade *et al*. (2013) obtained 24.9% *Acinetobacter* isolates from various clinical specimens. In the current study prevalence rate of *P. aeruginosa* was 20.9% which corroborates with previous studies where its prevalence was 10.5 to 30 % (Mehta *et al.,* 2001; Mohanasoundarm,

2011). Our data demonstrated 13.6 % *K. pneumoniae* which is in accordance with the results of Kumar and Kalpana (2013) where they noted prevalence of *K. pneumoniae* to be 14.5%. Frequency of isolation of pathogenic organisms from various specimens is depicted in Table 2. Antibiogram profile of all Gram negative pathogens recovered from clinical samples is shown in Figures 1 and 2. In this study, Potentox appeared as most efficacious agent with susceptibility rate 89.9, 86.6, 88, 89, 91.2 and 91.7 % to *E. coli*, *P. aeruginosa*, *A. baumannii*, *K. pneumoniae*, *Citrobacter species* and *Proteus species* respectively, which was high compared to other tested drugs. Potentox synergistically is reported of having protein kinase inhibitor activity to inhibit the aminoglycoside modification through ATP-dependent O-phosphorylation, catalysed by aminoglycoside kinases particularly aminoglycoside phosphotransferases (Aphs). Its antibacterial activity has also been proved in animal model (Chaudhary *et al.,* 2011; Dwivedi *et al.*, 2009).

A very high level of resistance to monotherapies were observed where cefepime was 55%-74%, tobramycin 20.8%-70.2%, gentamycin 40%-65.6%, levofloxacin 32.9%-62.4%, moxifloxacin 40.1%-47.7%, ofloxacin 29.9%-48.6%, imipenem plus cilastatin 30%-60%, ciprofloxacin 52.3%-75.7%, ceftazidime 50.3%-60%, azithromycin 40.5%-86%, and amikacin 40.4%-79.7% among all isolated Gram negative bacterial pathogens. Potentox (cefepime+amikacin) showed very low resistance (8.2%-13.4%) among all Gram-negative pathogens (Figure 2). Khalili *et al.* (2012) observed the resistance rate of Gram- negative bacilli to cefepime 60, 67.9, 37.9 and 50% in 2007, 2008, 2009 and 2010, respectively in Iran. Other studies observed 51 to 84 % resistance to cefepime in Gram-negative organisms (Jazani *et al.,* 2010; De Macedo and Santos, 2005). A similar study performed by Aliakbarzade *et al.* (2014) reported that *Acinetobacter* spp. showed differential resistance pattern to aminoglycosides i.e. gentamicin (86%), tobramycin (63%) and amikacin (81%). Resistance to gentamicin and ciprofloxacin against *E. coli* observed in this study was comparable to a study by Yismav *et al.* (2010). Rajat *et al.* (2012) reported that *P. aeruginosa* isolated from various samples are resistant to tobramycin (68%) followed by gentamycin (63%), ciprofloxacin (49%) and ceftazidime (43%). Mandal *et al.* (2010) also showed that 44.5% of *Pseudomonas spp.* isolates were found to be resistant to ceftazidime which is similar to that reported in the current study where 50.3% *P. aeruginosa* isolates were resistant to ceftazidime. In our study when the activity of cefepime/amikacin (Potentox) was compared with that of eleven other antibiotics including cefepime and amikacin alone, the susceptibility for all pathogens were significantly higher for Potentox.

Conclusion

The bacterial susceptibility and resistance profile of all isolates in this study have shown that Potentox remain the most effective drugs against Gram negative pathogens, suggesting that use of it over other antibiotics should be preferred. However there is a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of reserve drugs to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

REFERENCES

Aliakbarzade, K., Farajnia, S., Nik, A.K., Zarei, F. and Tanomand, A., 2014. Prevalence of aminoglycoside resistance genes in *Acinetobacter baumannii* isolates. *Jundishapur J. Microbiol.,* 7: e11924.

Bhadade, R. 2013. Prospective evaluation and mortality outcome of nosocomial infections in medical intensive care unit at tertiary care teaching centre in Mumbai. *Emergency Med.,* 3: 159.

Bahashwan, S.A. and Shafey, H.M. 2013. Antimicrobial resistance patterns of *Proteus* isolates from clinical specimens. *Eur. Sci. J.,* 9: 188-202.

Chen, C.C., Lin, Y.C., Sheng, W.H., Chen, Y.C., Chang, S.C., Hsia, K.C., Liao, M.H. and LI, S.Y. 2011. Genome sequence of a dominant, multidrug-resistant *Acinetobacter baumannii* strain, TCDC-AB0715. *J. Bacteriol.,* 193:2361-2.

Chaudhary, M., Payasi, A. and Dwivedi, V.K. 2011. Comparative safety evaluation of potentox® vs co-administration of cefepime and amikacin in healthy albino rat. *Int. J. Drug Dev. Res.,* 3: 348-355.

Clinical and Laboratory Standards Institute, (2013) Performance standards for antimicrobial susceptibility testing; twenty-first informational supplement. CLSI document M100-S23. Wayne, PA.

Cheesbrough, M. 2000. Biochemical tests to identify bacteria. In: District laboratory practice in tropical countries. 2nd ed. Cambridge University Press, UK,178-187.

de Macedo, J.L. and Santos, J.B. 2005. Bacterial and fungal colonization of burn wounds. *Mem. Inst. Oswaldo. Cruz.* 100: 535-9.

Dwivedi, V.K., Chaudhary M., Soni A. and Shrivastava, S.M. 2009. Nephrotoxicity reduction by fixed dose combination of cephalosporins and aminoglycosides in Mus musculus mice. *Asian J. Biochem.,* 4: 13-21.

Doi, Y. and Y. Arakawa, 2007. 16S Ribosomal RNA Methylation: Emerging Resistance Mechanism against Aminoglycosides. *Clin. Infect. Dis.,* 45:88–94.

Gdalhoff, A. 2012. Global fluoroquinolone resistance epidemiology and implictions for clinical use. Inter. Persp. Infect. Dis., 2012:976273.

Dua, R.S. Shrivastava, S.K., Sonwane and Srivastava, S.K. 2011. Pharmacological significance of synthetic heterocycles scaffold: a review. *Adv. Biol. Res.,* 5: 120-144.

Galimand, M., Courvalin P. and Lambert, T. 2012. RmtF, a new member of the aminoglycoside resistance 16S rRNA N7 G1405 methyltransferase family. *Antimicrob.Agents Chemother.,* 56: 3960–3962.

Gad, G.F., Mohamed, H.A. and Ashour, H.M. 2011. Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients in upper Egypt. *PLoS ONE* 6: e17224.

Hughes, W.T., Armstrong, D., Bodey, G.P., Brown, A.E., Edwards, J.E., Feld, R., Pizzo, P., Rolston, K.V., Shenep J.L. and Young, L.S. 1997. Guidelines for the use of

antimicrobial agents in neutropenic patients with unexplained fever. *Clin. Infect. Dis.,* 25: 551-73

Hopkins, K.L., Wootton, L., Day, M.R. and Threlfall, E.J. 2007. Plasmid-mediated quinolone resistance determinat *qnrs1* found in *Salmonella enterica* strains isolotad in the UK. *J. Antimicrob. Chemother.,* 59: 1071-1075.

Jacoby, G.A., Walsh, K.E., Mills, D.M., Walker, V.J., Oh, H., Robicsek, A., Hooper, D.C. 2006. qnrB, another plasmid-mediated gene for quinolone resistance. *Antimicrob. Agents Chemother.,* 50: 1178-1182.

Jazani, N.H., Babazadeh, H., Sabah, Z., Zartoshti, M. 2010. The evaluation of antibiotic resistance to cefepime in hospital isolates of *Pseudomonas aeruginosa. J. Med. Biomed. Sci.,* 9: 17.

Kim, S.L., Jeong J.-Y., Jun J.-B., Choi S.-H., Lee, S.-O., Kim, M.-N., Woo, J.H. and Kim, Y.S. 2009. Prevalence of aac(6′)-Ib-cr encoding a ciprofloxacin-modifying enzyme among Enterobacteriaceae blood isolates in Korea. *Antimicrob. Agents Chemother.,* 53: 2643–2645.

Kollef, M.H. 2003. The importance of appropriate initial antibiotic therapy for hospital-acquired infections. *Am. J. Med.,* 115:582-584.

Kumar, A.R. and Kalpana, S. 2013. Prevalence and Antimicrobial Susceptibility Pattern of *Klebsiella Pneumoniae* Causing Urinary Tract Infection and issues related to the Rational S election of Antimicrobials. *Sch. J. App. Med. Sci.,* 1:395-399.

Khalili, H., Dashti-Khavidaki, S., Shahidi, M.R., Abdollahi, A., Jafari, S., Jahangard-Rafsanjani Z. and A.H. Talasaz, 2012. Changes in gram negative microorganisms' resistance pattern during 4?years period in a referral teaching hospital; a surveillance study. *J. Pharmaceut. Sci.,* 20: 28.

Lee, Y.-J,, Liu, H.-Y., Lin, Y.-C., Sun, K.-L., Chun, C.-L. and Hsueh, P.-R. 2010. Fluoroquinolone resistance of *Pseudomonas aeruginosa* isolates causing nosocomial infection is correlated with levofloxacin but not ciprofloxacin use. *Int. J. Antimicrob. Agents,* 35: 261-264.

Lautenbach, E, Strom, B.L., Bilker, W.B., Patel, J.B., Edelstein, P.H. and Fishman, N.O. 2001. Epidemiological investigation of fluoroquinolone resistance in infections due to extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae. *Clin. Infect. Dis.,* 33**:**1288-1294.

Mandal, J., Srinivas, Acharya, N., Buddhapriya D. and Parija, S.C. 2012. Antibiotic resistance pattern among common bacterial uropathogens with a special reference to ciprofloxacin resistant *Escherichia coli.* Indian J. Med. Res., 136: 842-849.

Murdoch, D.R., Mirrett, S., Harrell, L.J., Donabedian, S.M., Zervos, M.J. and Reller, L.B. 2003. Comparison of Microscan Broth Microdilution, Synergy Quad Plate Agar Dilution, and Disk Diffusion Screening Methods for Detection of High-Level Aminoglycoside Resistance in Enterococcus Species. *J. Clin Microbbiol.,* 41; 2703–2705.

Miró, E., Grünbaum, F., Gómez, L., Rivera, A., Mirelis, B., Coll, P. and Navarro, F. 2013. Characterization of aminoglycoside-modifying enzymes in Enterobacteriaceae clinical strains and characterization of

the plasmids implicated in their diffusion. *Microb. Drug Res.,* 19: 94-99.

McDonald, L.C., Chen, F.J., Lo, H.J., Yin, H.C., Lu, P.L., Huang, C.H., Chen, P., Lauderdale, T.L. and Ho, M. 2001. Emergence of reduced susceptibility and resistance to fluoroquinolones in *Escherichia coli* in Taiwan and contributions of distinct selective pressures. *Antimicrob. Agents Chemother.,* 45:3084-3091.

Mehta, M., Bhardwaj, S., Sharma, J. 2012. Prevalence and antibiotic susceptibility susceptibility pattern of multi-drug resistant *E. coli* isolates from urinary tract infection (UTI) patients. Int. J. Life Sci. Pharma Res., 2: L-6-L-11.

Mohanasundaram, K. M. 2011. The antimicrobial resistance pattern in the clinical isolates of Pseudomonas aeruginosa in a tertiary care hospital: 2008-2010 (a 3 year study). *J. Clin. Diag. Res.,* 5: 491-94.

Nayar, R., Shukla, I. and Sultan, A. 2014. Epidemiology, prevalence and identification of *Citrobacter* species in clinical specimens in a tertiary care hospital in India. *Int. J. Sci. Res.,* 4: 1-6.

Poole, K. 2004. Efflux-mediated multi resistance in Gram-negative bacteria. *Clin. Microbiol. Infect.,* 10:12-26

Robicsek, A., Jacoby, G.A. and Hooper, D.C. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *The Lancet infect. Dis.,* 6: 629-640.

Randhawa, V.S., Kapoor, L., Singh, V. and Mehta, G. 2004. Aminoglycoside resistance in *Enterococci* isolated from pediatric septicaemia in a tertiary care hospital in North India. *Indian J. Med. Res.,* 119: 77-79

Rajan, S. and Prabavathy, J. 2012. Antibiotic sensitivity and phenotypic detection of ESBL producing *E. coli* strains causing urinary tract infection in a Community Hospital, Chennai, Tamil Nadu, India. *Cen. Pharmaceut. Sci.,* 3: WMC003840.

Shakil, S., Khan, R., Zarrilli, R. and Khan A. U. 2008. Aminoglycosides versus bacteria—a description of the action, resistance mechanism, and nosocomial battleground. *J. Biomed. Sci.,* 15:5-14.

Solomkin J. S., Mazuski, J. E., Bradley, J. S. et al., 2010. "Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the the Surgical Infection Society and the Infectious Diseases Society of America". Clin. Infect. Dis., 50:133–164.

Shahid, M. and Malik, A. 2005. Resistance due to aminoglycoside modifying enzymes in *Pseudomonas aeruginosa* isolates from burns patients. Indian J. modifying enzymes detected in strains of Med. Res., 122: 324-32

Sanz, M.A., Lopez, J., Lahuetra, J.J., Rovira, M., Batlle, M., Perez, C., Vazquez, L., Julia, A. , Palau, J., Gutierrez, M., Capote, F.J., Ramos, F., Benlloch, L., Larrea, L. and Jarque, I. Spanish PETHEMA group. 2002. Cefepime plus amikacin versus piperacillin tazobactam plus amikacin for initial antibiotic therapy in haematology patients with febrile neutropenia results of an open, randomized, multicentre trial. *J. Antimicrob. Chemother.,* 50: 79-88.

Shafiyabi, S., Krishna, S., Jeer, M., Pavithra, Divya. 2014. Trends in antibiotic resistance pattern among escherichia coli isolates from patients with urinary tract infection in

tertiary care Hospital, Bellary. *Int. J. Pharm. Sci. Rev. Res.,* 24: 43-49

Tran, J.H., Jacoby, G.A. and Hooper, D.C. 2005. Interaction of the plasmid encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrob. Agents Chemother.,* 49: 118-125.

Wu, J.J., Ko, W.C., Tsai, S.H. and Yan, J.J. 2007. Prevalence of plasmid-mediated quinolone resistance determinants QnrA, QnrB, and QnrS among clinical isolates of *Enterobacter cloacae* in a Taiwanese hospital. *Antimicrob. Agents Chemother.,* 51:1223-1227.

Wang, Y., Ha, U., Zeng, L. and Jin, S. 2003. Regulation of membrane permeability by a two-component regulatory system in *Pseudomonas aeruginosa. Antimicrob. Agents Chemother.,* 47: 95–101.

Workowski, K. A. and Berman, S. 2010. "Sexually transmitted diseases treatment guidelines, 2010," Morbidity and Mortality Weekly Report, 59:1–113.

Xiong, Z.Z., Zhu, D.M., Wang, F., Zhang, Y.Y., Okamoto, R. and Inoue, M. 2002. Investigation of extended-spectrum β-lactamase in Klebsiellae pneumoniae and Escherichia coli from China. *Diagn. Microbiol. Infect. Dis.,* 44**:**195-200.

Yang, J., Luo, Y., Li, J., Ma, Y., Hu, C., Jin, S., Ye, L. and Cui, S. Characterization of clinical *Escherichi coli* isolates from china containing transferable quinolone resistance determinants. *J. Antimicrob. Chemother.,* 65: 435-459.

Yismaw, G., Abay, S., Asrat, D., Yifru, S. and Kassu, A. 2010. "Bacteriological profile and resistant pattern of clinical isolates from pediatric patients, Gondar University Teaching Hospital, Gondar, Northwest Ethiopia". Ethiopian Med. J., 48293–300.

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