http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (12): 2051-2054, 2004
ISSN 1028-8880

Isolation, Identification and Antibiotic Resistance Profile of Indigenous Bacterial Isolates from Urinary Tract Infection Patients

Nadia Gul, Talat Y. Mujahid and Samia Ahmad
Department of Microbiology, University of Karachi, Karachi-75270, Pakistan

Abstract: Sixty-five bacterial strains were isolated from urine samples of patients suffering from urinary tract infection and identified by conventional methods. Eighty percent of total isolated organisms were found to be gram negative while remaining 20% were gram positive. Among gram negatives, *E. coli* and gram positive *S. aureus* and *S. pyogene* were the most prevalent organism. The percentage of gram negative isolates was as follows *Escherichia coli* (47.6%) followed by *Pseudomonas aeruginosa* (9.2%), *Klebsiella pneumoniae* (7.6%), *Enterobacter aerogenes* (6.1%), *Proteus mirabilis* and *Serratia marcescens* (4.6% each). The percentage of gram positive isolates includes, *Staphylococcus aureus* and *Streptococcus pyogenes* (4.6% each), *Enterococcus faecalis, Staphylococcus epidermidis* and *Bacillus subtilis* (3% each) and *Staphylococcus saprophyticus* (1.5%). The antibiotic resistance of identified organisms was carried out by disc-diffusion method with commercially available disc of fifteen antibiotics having different mode of actions such as cell wall synthesis inhibitors, membrane permeability alternatives, protein synthesis inhibitors and DNA synthesis inhibitors. Gram negatives showed more resistance to these antibiotics as compared to gram positive organisms. The most effective antibiotic for gram negative UTI isolates is gentamycin showing 69.2% efficacy, then sulfamethoxazole-trimethoprim (SXT-TMP) with 55% efficacy and then kanamycin having 50% efficacy. Among gram positives, chloramphenicol is most effective with 84.6% efficacy, then ofloxacin and gentamycin with 76.9% efficacy and then norfloxacin with 69.2% efficacy.

Key words: Urinary tract infection, antibiotics, antibacterial, resistance, sensitive

INTRODUCTION

Urinary Tract Infections (UTIs) are among the most common bacterial infections ranging from asymptomatic to severe sepsis[1]. It also contributes the most common nosocomial infection in many hospitals and accounts for approximately 35% of all hospital-acquired infections. UTI cause increased morbidity and economic cost[2]. Bacteria are the primary organisms that cause UTI. Gram negatives cause 80-85% and gram positives cause 15-20%. Among, gram negatives *Escherichia coli* is the most frequent pathogen[3] but in complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella, Proteus, Serratia, Enterobacter, Pseudomonas.* Among gram positives *S. saprophyticus, E. faecalis, S. agalactiae, S. pyogenes, S. aureus* and *Bacillus subtilis* are usually prevalent and are resistant to a variety of different antibiotics[4]. *Enterococcus isolates* cause 2.3% of UTI and best known as antibiotic resistant opportunistic pathogens[5]. Varieties of antibiotics are available for UTIs and the choice is depend upon many factors including severity of infection and primary or recurrent infection. Antibiotics used for treatment of UTI includes amoxycillin, cephalosporins, tetracycline, nitrofurantoin but fluoroquinolones are most commonly used[6]. For catheter induced infection antibiotics in combinations are effective against a wide variety of microorganisms includes fluoroquinolones, ampicillin+Gentamicin, imipenem+cilastatin[7].

Development of resistant strains is a common problem in antimicrobial chemotherapy. Among uropathogens the rate of resistance is high and frequency of resistance to antibiotics and drugs is directly linked to consumption of antibiotics[8]. According to Tomasz[9] gram positive bacteria are the most common cause of nosocomial infections. Enterococci are best-known antibiotic resistant opportunistic pathogens, resistant to vancomycin[5]. No single antibiotic is bactericidal for Vancomycin Resistant Enterococci (VRE) and Methicillin-Resistant *Staphylococcus aureus* (MRSA) and combination therapy is mandatory[10]. Present study was based on the isolation-identification and antibiotic resistance pattern of about sixty-five indigenous bacterial isolates from UTI patient.

Corresponding Author: Samia Ahmad, Department of Microbiology, University of Karachi, Karachi-75270, Pakistan
Tel: 03002343201 E-mail: samiaz@gerrys.net

MATERIALS AND METHODS

Isolation and identification of UTI isolates: Sixty-five urine samples from UTI patients were collected from different pathological laboratories of cosmopolitan city of Karachi. For the isolation of UTI causing strains, loop full of urine sample was streaked on to nutrient agar plate and incubated at 37°C for 24 h. Next day individual colonies were selected and identified on the bases of morphological, cultural and biochemical characteristics[11].

For identification of gram negative bacteria: To check morphological characteristics, gram-staining, capsule staining (Anthony's method) and motility test were performed. To check the growth pattern, different media including MacConkey's agar no.3, Eosine Methylene Blue agar (BioM laboratories, USA) were used. For biochemical characteristics, sugar fermentation (lactose, glucose, mannitol, maltose, sucrose and xylose), TSI, IMVIC (indole, MR, VP, citrate) and nitrate tests were performed[4,11].

For identification of gram positive bacteria: To check morphological characteristics, gram-staining and capsule staining (Anthony's method) was performed. To check the growth pattern, different media including MacConkey's agar no.3, Nutrient agar, Brain Heart Infusion agar, Mannitol Salt agar and blood agar base (Oxoid) supplemented with 5% sheep blood were used. For biochemical characteristics, sugar fermentation, oxidase, catalase, coagulase, novabiocin, optochin, bacitracin and bile esculin sensitivity test were performed[4,11].

Maintenance of clinical isolates: Stock cultures were maintained in vials by growing the UTI isolates in 3 mL nutrient broth and next day overlaying with 3 mL 40% glycerol[12]. Vials were then freezed at -70°C.

Determination of antibiotic resistance profile: UTI isolates were subjected to antibiotic resistance screening by disc diffusion method. For this purpose, lawn of UTI isolates was made on nutrient agar plates with the help of wire-loop. Then commercially available antibiotic discs were placed on lawn of culture and plates were incubated at 37°C for 24 h. Next day presence or absence of zone of inhibition around the antibiotic discs was observed[11]. Antibiotics used were penicillin G and amoxicillin (cell wall synthesis inhibitors), polymyxin B (membrane permeability alternatives), tetracycline, kanamycin, tobramycin, neomycin, gentamycin, chloramphenicol, ethyl-hydrocupreine hydrochloride (optochin) and lincomycin (protein synthesis inhibitors), ciprofloxacin, norfloxacin, sulfamethoxazole-trimethoprim (SXT-TMP) and ofloxacin (DNA synthesis inhibitors).

RESULTS AND DISCUSSION

Urinary Tract Infections (UTIs) are among the most commonly prevalent infections in clinical practice. The purpose of the present study is to describe the susceptibility or resistance profile of multi-drug resistant isolates from urinary tract infection. Sixty-five isolates from different pathological laboratories of Karachi (Pakistan) were isolated and identified by conventional methods. Identification of the causative organism and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages of UTI[13]. Percentage of different gram negative and gram positive isolates in urinary tract infections is depicted in Table 1. The frequency of gram negative enteric bacteria causing UTI is more then gram positives. Among gram negatives, *E. coli* was the most predominant organism. The percentage of gram negative isolates was as follows *Escherichia coli* (47.6%) followed by *Pseudomonas aeruginosa* (9.2%,) *Klebsiella pneumoniae* (7.6%), *Enterobacter aerogenes* (6.1%), *Proteus mirabilis* and *Serratia marcescens* (4.6% each). The percentage of gram positive isolates includes, *Staphylococcus aureus* and *Streptococcus pyogenes* (4.6% each), *Enterococcus faecalis, Staphylococcus epidermidis* and *Bacillus subtilis* (3% each) and *Staphylococcus saprophyticus* (1.5%). Present results are in close agreement with Ali[14] which reported organisms responsible for UTI include *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus* spp. and *Pseudomonas aeruginosa*. Previous studies have also demonstrated that *E. coli* is the most frequent etiological agent causing community and hospital acquired UTIs[3,15-17].

All the isolates were screened for drug resistance profile by disc-diffusion method with commercially available disc of penicillin G, amoxicillin (cell wall synthesis inhibitors), polymyxin B (cell membrane alternative), gentamycin, kanamycin, neomycin, tetracycline, tobramycin, chloramphenicol, ethyl-hydrocupreine hydrochloride (optochin) and lincomycin (protein synthesis inhibitors), ciprofloxacin, ofloxacin, sulfamethoxazole-trimethoprim (SXT-TMP) and norfloxacin (DNA synthesis inhibitors). Table 2 indicates the resistance level against commonly used antibiotics in urinary tract infection. Nearly all the isolates (gram positive and gram negative) were found to be resistant against most of the antibiotics, whereas gram negative shows more resistance as compare to gram positive

Table 1: Percentages of gram negative and gram positive bacteria isolated from UTI

Bacterial isolates	Total number organism	Total % of UTI isolates	Individual % of gram negative and gram positive UTI isolates
Gram negative	**52**		
E. coli	31	47.6	60
K. pneumoniae	5	7.6	9
E. aerogenes	4	6.1	7
P. mirabilis	3	4.6	6
S. marcescens	3	4.6	6
P. aeruginosa	6	9.2	12
Gram positive	**13**		
S. aureus	3	4.6	23
S. epidermidis	2	3.0	15
S. saprophyticus	1	1.5	8
S. pyogenes	3	4.6	23
E. faecalis	2	3.0	15
B. subtilis	2	3.0	15

Table 2: Total percentage efficacy of different antibiotics among UTI isolates

		Total No. of isolates (65)		
Antibiotic	Disc code	Sensitive	Resistant	% Efficacy
Penicillin G	P_G	2	63	3.0
Amoxicillin	A_{mx}	9	56	13.8
Polymyxin B	P_B	1	64	1.5
Gentamycin	G_{m10}	47	18	72.3
Kanamycin	K_{30}	36	29	55.3
Neomycin	N	8	57	12.3
Tetracycline	T_{25}	2	63	3.0
Tobramycin	T_{N10}	23	42	35.0
Chloramphenicol	C_{30}	37	28	56.9
Lincomycin	L_2	2	63	3.0
Ciprofloxacin	Cip	30	35	46.1
Norfloxacin	Nor	34	31	52.3
Ofloxacin	Ofx	31	34	47.6
Optochin	Op	0	65	0.0
Sulfamethoxazole-trimethoprim	Sxt	33	32	50.7

Table 3: Percentage effectiveness of different antibiotics against gram positive and gram negative UTI isolates

	Percentage effectiveness	
Antibiotic	Gram positive (%)	Gram negative (%)
Penicillin G	15.00	0.0
Amoxicillin	53.80	3.8
Polymyxin B	7.60	0.0
Gentamycin	76.90	69.2
Kanamycin	61.50	50.0
Neomycin	7.60	13.4
Tetracycline	7.60	1.9
Tobramycin	46.10	32.6
Chloramphenicol	84.60	50.0
Lincomycin	15.38	0.0
Ciprofloxacin	46.10	48.0
Norfloxacin	69.20	48.0
Ofloxacin	76.90	40.3
Optochin	0.00	0.0
Sulfamethoxazole-trimethoprim	30.70	55.7

organisms. Resistance to aminoglycosides and chloramphenicol in gram negative bacilli is often mediated by ß-lactamases which are unaffected by exposure of the bacterium to the potential drugs[18]. Interestingly, resistance to penicillin G, amoxicillin, polymyxin B, neomycin, tetracycline, tobramycin, lincomycin and optochin is more frequent in our study. It has been argued that there is a direct relation between the antibiotic used and the frequency and kinds of antibiotic-resistant strains in human beings[19]. The resistance to antimicrobial agents can readily be transferred among bacteria by transmissible elements/plasmids[20]. These resistant organisms can pass their resistance genes to their offspring by replication or to related bacteria through conjugation[9]. Epidemiological studies have suggested that antibiotic resistance genes emerge in microbial populations within 5 years of the therapeutic introduction of an antibiotic[21]. Further, the antibiotic resistance genes (found in human and animal isolates) could have originated in the industrial microbes that are used for the production of antibiotics[22]. In our studies, the most effective antibiotic for gram negative UTI isolates is gentamycin showing 69.2% efficacy, then sulfamethoxazole-trimethoprim (SXT-TMP), which shows 55% efficacy and then kanamycin showing 50% efficacy. Among gram positives, chloramphenicol is most effective with 84.6% efficacy, then ofloxacin and gentamycin with 76.9% efficacy and then norfloxacin with 69.2% efficacy (Table 3). Ciprofloxacin is highly active against UTI pathogens[23] and maintain excellent level of susceptibility among common UTI pathogens[24]. In the present study ciprofloxacin showed 46.1% efficacy against gram positives and 48.0% against gram negative bacteria. Due to frequent use susceptibility to ciprofloxacin decreases in a stepwise manner[25] and resistance rates increasing among, UTI isolates[26]. So wide spread empirical use of fluoroquinolones should be discouraged because of potential promotion of resistance[27]. Gram positive bacteria are also most frequent cause of nosocomial diseases and difficult to treat because of their high frequency of drug resistance[9]. Due to antibiotic resistance and limited antimicrobial activity of antibiotics some strains of disease causing bacteria are now untreatable such as vancomycin-resistance *Enterococcus*[28]. *Enterococcus faecalis* showed 100% sensitivity to amoxicillin and gentamycin and 50% to penicillin G while, resistant to rest of the antibiotics tested in this study (data not shown).

Hence present study shows that UTI pathogens/isolates showed decreased susceptibility to most of the antibiotics usually used for the treatment of UTI. It is now very necessary to develop new antimicrobials and therapeutic agents having high effectiveness with no side effects, easy availability and less expensive.

ACKNOWLEDGMENT

This research project was supported by Karachi University Grant (DFS-2003) to Ms. Talat Y. Mujahid.

REFERENCES

1. Hussain, N., 2001. Prevalance and susceptibility pattern of urinary pathogens. Biomedica, 18: 76-79.
2. Foxman, B., 2003. Epidemiology of urinary tract infections: Incidence, morbidity and economic costs. Dis. Mon., 49: 53-70.
3. Gales, A.C., H.S. Sader and R.N. Jones, 2002. Urinary tract infection trends in Latin American hospitals. Diagn. Microbiol. Infect. Dis., 44: 289-299.
4. Thomas, J.G., 1995. Urinary Tract Infections. In: Diagnostic Microbiology. (Eds. Mahon, C.R. and G. Manuselis), pp: 950-969.
5. Murray, B.E., 2000. Vancomycin-resistant enterococcal infections. The New England J. Medicine, 342: 710-721.
6. Weir, M. and J. Brien, 2000. Adolescent urinary tract infections. Adolesc. Med., 11: 293-313.
7. Williams, G.J., A. Lee and J.C. Craig, 2003. Antibiotics for the prevention of urinary tract infection in children: A systematic review of randomized controlled trials. J. Pediatrics, pp:46-50.
8. Goossens, H. and M.J.W. Sprenger, 1998. Community acquired infections and bacterial resistance. Bio. Med. J., 317: 654-657.
9. Tomasz, A., 1994. Multiple-antibiotic-resistant pathogenic bacteria-a report on the rockefeller university workshop. The New England J. Med., 330: 1247-1251.
10. Michel, M. and L. Gutmann, 1997. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant Enterococci: therapeutic realities and possibilities. Lancet, 349: 1901-1906.
11. Cheesbrough, M., 2000. Bacterial pathogens. In: District Laboratory Practice in Tropical Countries Vol. II. ELBS London, pp: 157-234.
12. Sambrook, J., E.F. Fritsch and T. Maniatis, 1989. Molecular cloning. A Laboratory Manual 2nd Edn. Cold Spring Harbor Laboratory Press. USA., pp: 1-440 .
13. Khan, M.T. and S.H. Shah, 1981. Experience with gram negative bacilli isolated from 400 cases of urinary tract infection (UTI) in Abbottabad. J. Ayub Med. Coll. Abbottabad, 12: 21-23.
14. Ali, N.S., 2000. Protocol for evaluation and management of urinary tract infection in adults. Pak. J. Med. Sci. Rev., 16: 251-254.
15. Brosnema, D.A., J.R. Adams, R. Pallares and R.P. Wenzel, 1993. Secular trends in rates and etiology of nosocomial urinary tract infections at a university hospital. J. Urol., 150: 414-416.
16. Weber, G., K. Riesenberg, F. Schlaeffer, N. Peled, A. Borer and P. Yagupsky, 1997. Changing trends in frequency and antimicrobial resistance of urinary pathogens in outpatient clinics and a hospital in Southern Israel. Eur. J. Microbiol. Inf. Diseases, 16: 834-838.
17. Noor, N., M. Ajaz, S.A. Rasool and Z.A. Pirzada, 2004. Urinary tract infections associated with multi drug resistant enteric bacilli: characterization and genetical studies. Pak. J. Biol. Sci., 17: 115-123.
18. Shafran, S.D., 1990. The basis of antibiotic resistance in bacteria. J. Tolaryngol., 19: 158-168.
19. Kupersztoch, P.Y.M., 1981. Antibiotic resistance of gram negative bacteria in Mexico: Relationship to Drug Consumption. In: Molecular Biology, Pathogenicity and Ecology of Bacterial Plasmids (Levy, S.B., R.C. Clowes and E.L. Koenig, Eds.). Plenum Press, New York, pp: 529-537.
20. Neu, H.C., 1994. Emerging trends in antimicrobial resistance in surgical infections. A review. Eur. J. Surg., 573: 7-18.
21. Chakrabarty, A.M., S.G. Dastidar, M. Ganguli and C. Chattopadhayay, 1990. DNA as contaminants in antibiotics and its capacity to transform bacteria to drug resistance. Indian J. Exp. Biol., 28: 58-62.
22. Web, V. and J. Davis, 1993. Antibiotic preparations contain DNA: A source of drug resistance genes. Antimicrob. Agents. Chemother., 37: 2379-2384.
23. Farrell, D.J., I. Morrissey, D. Rubeis, M. Robbins and D. Felmingham, 2003. A UK. Multi center study of the antimicrobial susceptibility of bacterial pathogens causing urinary tract infection. J. Infec., 46: 94-100.
24. Gupta, K., D. Scholes and W.E. Stamm, 1999. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women. JAMA., 281: 736-738.
25. Karlowsky, J.A., M.E. Jones and C. Thornsberry, 2003. Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. Antimicrob. Agents. Chemother., 47: 1672-1680.
26. Gales, A.C., R.N. Jones, K.A. Gordon, H.S. Sader, W.W. Wilke, M. Beach, L. M.A. Pfaller and G.V. Doern, 1998. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: Report from the second year of the sentry Antimicrobial Surveillance Program (1998). J. Antimicrob. Chemother., 45: 295-303.
27. Nicolle, L., 2003. Best pharmacological practice: Urinary tract infection. Expert Opin. Pharma., 4: 693-704.
28. Hancock, L.E. and M.S. Gilmore, 2000. Pathogenecity of Enterococci. Published In: Gram Positive Pathogens. A.S.M. Publications, pp: 251-258.