In-Vitro Activity of Nitrofurantoin in Enterococcus Urinary Tract Infection

T. Butt (Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi.)
M. J. Leghari (Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi.)
A Mahmood (Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi.)

Introduction

Urinary Tract Infections (UTIs) are a global health problem and are the second most common infection after the respiratory tract infections. In United States, they account for approximately 8 to 10 million physician visits annually and one fifth of emergency department visits per year.1,2 UTIs are the leading cause of Gram-negative bacteremia in patients of all ages and are associated with a high rate of morbidity and mortality, especially in the elderly.3 The most common uropathogens identified in adult patients with UTIs are the enteric Gram negative rods with Escherichia coli being the most frequent (60-80%). Enterococci account for approximately 5% of UTIs and most of these are nosocomial.4 An estimated 110,000 UTIs are caused by the enterococci annually in the United States.5 The true incidence of UTIs caused by enterococci in Pakistan is not known. According to a study carried out in Karachi, estimated frequency of enterococcal UTIs is about 2%.6

MacCallum and Hastings initially addressed enterococcus pathogenicity at the end of 19th century.7 Infection caused by the genus Enterococcus (most notably Enterococcus faecalis, which accounts for 80% of all enterococci infections) include urinary tract infection, bacteremia, intrabdominal infections and endocarditis.5

In recent years enterococci have emerged as important nosocomial pathogens. The most important characteristics of enterococci are their intrinsic resistance to several antimicrobial agents and their ability to acquire resistance. The rapid emergence of antimicrobial resistance among enterococci, undoubtedly also contributes to their emergence as prominent nosocomial pathogens making them among the most difficult to treat. Inherent in vivo resistance of Enterococcus faecalis to trimethoprim-sulfamethoxazole may explain the lack of efficacy of animal models. In vitro, trimethoprim-sulfamethoxazole readily inhibits most enterococci at low concentration but this activity is lessened by exogenous folates.8

The Enterococci are now receiving increased attention because of their resistance to multiple antimicrobial drugs. The most common enterococci associated nosocomial infections are infection of the urinary tract followed by surgical site infection and bacteremia.9-11 Resistance of the enterococci against ß-lactams, macrolides, aminoglycosides and glycopeptides is on increase. As the resistance of Enterococcus to the commonly used antimicrobials is high, therefore the effectiveness of other antimicrobial agents against Enterococcus spp. was evaluated while keeping in view the cost effectiveness of antimicrobial therapy. Nitrofurantoin (NIT) has been in use for several decades in clinical practice. There is consistently low level of resistance to NIT against the microorganisms responsible for urinary tract infections. Gram positive cocci as well as Gram negative bacilli are susceptible to NIT with few exceptions. Seven days regimen may be required to achieve a higher rate of cure as compared to three days regimen.12-14 NIT is bacteriostatic and bactericidal and among susceptible populations of microorganisms, resistant mutants are rare. Clinical drug resistance emerges slowly. There is no cross resistance between NIT and other antimicrobial agents. Its activity is greatly enhanced at pH 5.5 or below.15,16 Various studies have indicated that Enterococcus faecalis and E. faecium are uniformly susceptible to NIT including most of the vancomycin resistant enterococci.17 In vitro antimicrobial susceptibility pattern of enterococci isolated from UTI cases, against all common antibiotics including NIT was planned to determine and to compare in vitro efficacy of NIT with other commonly used antibiotics. This would help in estimating the status of NIT in UTI particularly caused by enterococci and to use this cheap drug in clinical practice.

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Methods

A total of ~144 Enterococci (Enterococcus faecalis n=130, Enterococcus faecium n =14) among ~4007 urinary isolates from patients having signs and symptoms of urinary tract infections were included in the study. They were identified by Gram staining and biochemical profile using API 20 strept system SA, Montalieu Vercieu, France and conventional biochemical tests at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP) Rawalpindi from September 2000 to June 2003.

All the urine specimens from patients having signs and symptoms of urinary tract infections were collected and the urine specimens having pus cells (more than 4 cells per high power field from un-centrifuged urine) on microscopy were included. Patients were selected by convenience non probability sampling. All the specimens that were passed at the laboratory or received from the admitted patients, irrespective of age, sex or antibiotic therapy, were included. The urine specimens were excluded from the study, if the urine was passed at home or somewhere else, when the safe transport could not be ascertained, or if there were growth of more than one microorganism on culture.

A structured proforma was used for the collection of data. It demonstrated name and identification number of the patient, age, gender, urinary symptoms and signs, admitted or out patients and urine microscopy. Culture and sensitivity results were also endorsed on the proforma.

Mid stream urine samples were collected in a sterile screw capped containers. The samples were sent to the Department of Microbiology within an hour of collection. If there was any delay the specimens were refrigerated.

Urine microscopy was done for pus cells and was cultured with semi-quantitative dipstrip method (MAST BACTERURITEST) on Cystine Lactose Electrolyte Deficient (CLED) agar and incubated aerobically at 37oC for 24 hours. Any significant growth (The bacteriuria dipstrip is so designed that it picks and inoculate 0.2 µl of urine on the plate, therefore 20 or more colonies indicate the bacterial count as >105 CFU/ml) obtained was identified with colony morphology, Gram staining, catalase production, growth in nutrient broth containing 6.5% NaCl, aesculin hydrolysis in the presence of 40% bile salts and biochemical profile.

Antimicrobial susceptibility testing of the isolates was carried out using modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar as recommended by NCCLS.18 Staphylococcus aureus ATCC 25923 (for disc diffusion) and Enterococcus faecalis ATCC 29212 (for broth dilution) were used as control organisms. The disc used were nitrofurantoin (NIT) 300mg, doxycycline (DOX) 30 mg, co-trimoxazole (COT) 1.25/23.75 mg, ampicillin (AMP) 10 mg, imipenem (IPM) 10 mg and vancomycin (VAN) 30 mg. Isolates were interpreted as susceptible or resistant according to the sensitivity zones of the particular antimicrobial as recommended by the NCCLS.18 The isolates resistant to VAN on disc diffusion were confirmed by using VAN 6mg screening agarplate, MIC determination by microdilution technique (<4ug/ml) taken as susceptible and >32ug/ml as resistant).19,20

Results

A total of ~144 Enterococci (3.59% of the total organisms, n=4007) isolated in the period under study; Enterococcus faecalis were ~130 and Enterococcus faecium were ~14. However, overall, Escherichia coli was the most common organism (n=2014; 50.26%) isolated (Table 1).

Ages of the patients were between 7 to 90 years. Mean age being 44.76 years while median age was 39.50 years. Out of ~144 patients ~42 (29.17%) were indoor while ~102 (70.83%) were outdoor patients. Male and female patients were 43.75% (n=63) and 56.25% (n=81) respectively.

Although the isolates of enterococci revealed best susceptibility against IPM (91.66%) and VAN (98.33%), but NIT also had encouraging results. Twenty eight percent (n=41) of the enterococci were resistant to AMP. Among these ~41 AMP resistant enterococci, ~6 were Enterococcus faecium. NIT susceptibility (88.33%) was much better than that of other commonly used antibiotics. All the isolates were found susceptible to VAN except for ~2 isolates; these ~2 were Enterococcus faecium (Table 2).

NIT showed better susceptibility results as compared to AMP (p=0.0004), COT (p<0.0001) and DOX (p<0.0001). There was no significant difference between susceptibility against NIT and IPM. However, VAN susceptibility was better than NIT (p=0.00037) (Table 2).

Although there were ~41 isolates resistant to AMP but among them 78% (n=32) were susceptible to NIT. Similarly among the isolates resistant to COT, DOX and IPM, 64 to

Table 1. Distribution of isolates in urinary tract infextion (n=4007).

Causative microorganisms	Number of isolates %
Eausative microorganisms	2014 (50.26)
Enterococci	144(03.59)
Other's isolates	1849(46.15)

Others isolates include Klebsiella pneumoniae, K Oxytoca, Citrobacter freundii, Proteus mirabilis, Acinetobacter sp, Providentia sp, Pseudomonas aeruginosa.

Table 2. Antimicrobial susceptibility pattern of enterococci isolated from cases of urinary tract infection (n=144)

Antimicrobials	Number susceptible (%)
Nitrofurantion	127(88.19)
Ampicillin	103(71.53)
Cotrimoxazole	19(13.19)
Doxycycline	16(11.11)
Impienem	131(90.97)
Vancomycin	142(98.61)

88% were susceptible NIT. Even one of the two VAN resistant isolates was found susceptible to NIT (Table3).

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Discussion

Urinary tract infections are a major public health problem. Escherichia coli is the most common organism causing UTIs in community. In our study Escherichia coli (50.26%) was also the most common organism causing UTIs. Enterococci were responsible for UTIs in about 4% of the cases.

Table 3. Nitrofurantoin susceptibility of isolates otherwise resistant to other antimicrobials.

Antimicrobials	No.of resistant isolates	Isolates susceptible to Nitrofurantoin among resistant to other antimicro-bials (%)
Apicillin	41	32(78.05)
Cotrimoxazole	125	108(86.4)
Doxycycline	128	112(87.5)
Imipenem	11	7(63.64)
Vancomycin	2	1(50.00)

Urinary tract infections are more common in female and about 50% of women have an occurrence of UTI in their life time4 but in our study there was no significant difference in both sexes, perhaps it was due to the population of patients report to AFIP for investigations is mainly the male gender.

In a recent study, data compiled for approximately 15000 isolates over a 3 years period (1995-1997) showed that resistance to ampicillin and vancomycin was relatively uncommon among Enterococcus faecalis isolates (<2%).5 In our study resistance to ampicillin in Enterococcus faecalis was more marked (28.33%). This appears to be quite significantly high and is alarming. However, this finding is supported by a study, which showed that enterococci are highly resistance to ampicillin, ciprofloxacin, streptomycin, teicoplanin and vancomycin.5,14 Isolates from both outdoor and indoor patients in the present study were uniformly susceptible to nitrofurantoin. This is consistent with the findings of several studies. In one study in tertiary care hospital in Italy, more than 90% of the isolates of enterococci were found susceptible to Nitrofurantoin.21 Similar susceptibility was found in a recent multi-centre study at United States and Canada, which not only revealed that the enterococci are susceptible to linezolid, nitrofurantoin and chloramphenicol but also these antibiotics have been recommended for urinary tract infection caused particularly by vancomycin resistant enterococci.22 Inspite of some side effects, Nitrofurantoin has been used in the recent past successfully to eradicate Enterococci and other pathogens from the urinary tract.23 Recently because of development of resistant strains of enterococci, Nitrofurantoin has been recommended for treatment of UTI caused by enterococci susceptible or resistant to other antibiotics.22, 24, 25 Nitrofurantoin is bacteriostatic and bactericidal for many gram positive and gram negative bacteria and activity of nitrofurantoin is greatly enhanced at pH 5.5 or below. It is a cheap drug and can be given orally for months for the suppression of chronic urinary tract infection. The susceptibility pattern of enterococci against NIT is very encouraging and enterococci not only showed significantly very high susceptibility against NIT but most of the isolates, which were found resistant to other available antibiotics, were found susceptible to NIT. Although its major side effects of anorexia, nausea and vomiting may limit its use, but otherwise, it would be an effective and cheap antimicrobial in treatment of enterococcus urinary tract infection. However, it is to be noted that these findings are only laboratory based and the final proof or validation can be obtained by the use of NIT in clinical settings.

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Abstract

Objective: To compare in-vitro antimicrobial susceptibility of nitrofurantoin against commonly used antimicrobials for the treatment of urinary tract infection caused by enterococci.

Methods: A total of 144 enterococci (Enterococcus faecalis, n=130, Enterococcus faecium, n=14) isolated and confirmed by biochemical tests at the department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, for a period of three years were included in the study. The antimicrobial susceptibility tests of isolated organisms were performed by standard disc diffusion method as recommended by NCCLS. In addition to nitrofurantoin (NIT), doxycycline (DOX), co-trimoxazole (COT), ampicillin (AMP), imipenem (IPM) and vancomycin (VAN) were used for antimicrobial susceptibility testing.

Results: One hundred and twenty seven (88%) isolates of Enterococci were susceptible to NIT while 72% (n = 103) to AMP, 13% to COT, 11% to DOX, 91% to IPM and 99% to VAN. Among the strains resistant to AMP, COT, DOX, IPM and VAN, 50-88% were susceptible to NIT.

Conclusion: Nitrofurantoin is an effective antimicrobial in vitro and can be used for treatment of Enterococcus urinary tract infections (JPMA 54:466;2004).