



ANTIBIOTIC SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* CAUSING UTI REPORTED IN TERTIARY CARE CENTRE, TRIVANDRUM, KERALA, INDIA

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Abstract

Enterococci are the most common cause of urinary tract infections. The frequency of isolation of *Enterococci* from urinary tract of patients has risen. Vancomycin Resistant *Enterococci* along with other multidrug resistant bacteria are the main concerns for physicians. *Enterococci* may also act as vehicle for spread of resistant genes. The objective of this study was to determine the antimicrobial susceptibility pattern of *Enterococci spp.* isolated from urine samples. This cross-sectional study was conducted at Department of Microbiology Dr. Somervell Memorial CSI Medical College and Hospital, Karakonam, Trivandrum, Kerala, India. Thirty six *Enterococci* isolated from UTI cases were processed from August 2019 to October 2019. In-vitro drug-susceptibility tests of *Enterococci* isolates were performed on Mueller Hinton agar. A total of 36 urine specimens yielding growth of *Enterococci* were studied. Among these only 19.99% samples were from OPD, from the IP patients (80.56), out of that 24.14% from ICU's and rest were from wards (75.86%). Females (83.33%) were found to be more prone to Enterococcal infection as compared to males (16.67%). High prevalence of Enterococcal infection was seen in the age group 61-70 years (25%) followed by 51-60 and 71-80 (16.67%) age groups. Enterococcus strains showed 100% sensitivity against vancomycin and linezolid. 50% of the isolates showed resistant towards high level aminoglycosides. Apart from the above sensitivity pattern the urinary isolates were observed with Nitrofurantoin (88.88%), Ampicillin (55.55%), Penicillin (52.77%) and Norfloxacin (16.66%). In this study shows highly sensitive towards Nitrofurantoin. These data may aid health professionals in choosing the appropriate treatment for patients with Enterococcal UTI in the region and hopefully will prevent the misuse of antibiotics.

Key words: Antibiotic Susceptibility, *Enterococci*, UTI, Tertiary care centre, Kerala

Introduction

Urinary Tract Infection (UTI) is one of the most important causes of morbidity in the general population and is the second most common cause of morbidity among hospitalized patients. It has been estimated that symptomatic UTIs result in as many as 7 million visits to outpatient clinics, 1 million visits to emergency departments and 100,000 hospitalizations annually (Nisha and Ashalatha (2017); Wilson and Gaido, 2004).

Nowadays, it represents one of the most common diseases encountered in medical practice affecting people of all ages from the neonate to the geriatric age group (Kunin, 1994). Most infections are caused by retrograde ascent of bacteria from the faecal flora via the urethra to

the bladder and kidney especially in the females who have a shorter and wider urethra and is more readily transfer by microorganisms (Jones *et al.*, 2006). The structure of the females urethra and vagina makes it susceptible to trauma during sexual intercourse as well as bacteria been massaged up the urethra and into the bladder during pregnancy and or child birth (El-Sweih *et al.*, 2008; Kolawale *et al.*, 2009).

The risk of developing urinary tract infection increases significantly with the use of indwelling devices such as catheters and urethral stents or sphincters (Foxman, 2003). Urinary tract infections account for an estimated 25 to 40% of nosocomial infections and represent the most common type of these infections (Bagshaw and Laupland, 2006).

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Enterococci are Gram-positive cocci, which are normal commensals of the gastrointestinal tract, genital tract and anterior urethra. It is the second most common cause of urinary tract infection (UTI) and third most common cause of bacteremia (Cetinkaya *et al.*, 2000; Gordon *et al.*, 1992). In each year, millions of people are affected by urinary tract infection (UTI), it is a serious health problem. In the world, all age groups across the lifespan are affected and it is the most cause of mortality and morbidity (Foxman *et al.*, 2000). UTIs are the most common of the Enterococcal infections: Enterococcus species have been implicated in approximately 10% of all UTIs and in up to approximately 16% of nosocomial UTIs (Centers for Disease Control and Prevention (CDC), 1993).

Most frequent infections caused by *Enterococcus spp.* are urinary tract infections followed by intra-abdominal abscesses and bloodstream infections (Low *et al.*, 2001). In addition, studies have shown increasing resistance of *Enterococci* to anti-microbial agents such as β -lactams and high-level resistance to aminoglycosides and more recently to glycopeptides. This is possibly due to the use of broad-spectrum antibiotics or multi-antibiotic regimes, which allow for enterococcal over growth and super infection (Kaçmaz and Aksoy, 2005). β -lactams and aminoglycosides are generally the antibiotics of choice for treating the serious infections caused by *Enterococci*. High-level gentamicin-resistant (HLGR) enterococci frequently express additional resistance to multiple antibiotics, thereby causing therapeutic problems (Schouten *et al.*, 2000). A high mortality rate of Enterococcal infections is due to increasing resistance of the organism to β lactam antibiotics, aminoglycosides and glycopeptides and inadequate response to the treatment (Shah *et al.*, 2012).

The intrinsic resistance of *Enterococci* involves cotrimoxazole, aminoglycosides and cephalosporins which are commonly used to treat UTIs and other enterococcal infections. Acquired antimicrobial resistance is also important. It allows virulent *Enterococci* to survive for extended periods of time in hospital settings (Arias *et al.*, 2011). Moreover, acquired vancomycin resistance is transferable to other organisms such as *Staphylococcus aureus* and *Listeria monocytogenes* (Shinde *et al.*, 2012).

In almost all cases there is a need to start treatment before the final microbiological results are available. Area-specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to choose the right empirical treatment (Katarzyna *et al.*, 2001).

Materials and Methods

A hospital based cross-sectional study was conducted at Dr. Somervell Memorial C.S.I Medical College and Hospital, Karakonam, Trivandrum, Kerala, India. Urine samples were collected from the patients with the symptoms of UTI attended in both IP and OP of health care centre for the period between: August 2019 to October 2019. Midstream specimens of urine (MSU) were collected in labeled and sterile wide mouth plastic container (Tille *et al.*, 2014). After collection the specimens were transported to be analyzed in the laboratory of Microbiology Department. The samples were plated on Blood Agar (Microexpress, A Division of Tulip Diagnostics (P) Ltd. India) and Mac Conkey Agar ((Hi Media Laboratories Private Limited, Mumbai) media by the semi-quantitative plating method using the calibrated loop technique (0.001 mL). Plates were incubated aerobically overnight at 37°C. Pure growth of an isolate in a count of $\geq 10^5$ colony forming units (CFU) per milliliter of urine was considered as significant bacteriuria. Growth of ≥ 3 isolates in a sample was considered as contamination and a repeat sample was advised. Conventional methods of identification were used for identification of the bacterial isolates. (Baron *et al.*, 2002).

Antimicrobial sensitivity test was done on Mueller Hinton agar by the Kirby-Bauer technique according to the CLSI guidelines (CLSI-2007) using *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) as control strains. The antimicrobial agents used in antibiotic susceptibility testing were Penicillin (10 μ g), Erythromycin (15 μ g), Ampicillin (25 μ g), Linezolid (30 μ g), Vancomycin (30 μ g), Choloramphenicol (30 μ g) and High level Gentamicin (30 μ g) (Hi Media Laboratories Private Limited, Mumbai).

Result

A total of Thirty six *Enterococci* strains were isolated from clinically suspected UTI patients the study period. Among these only 19.44% isolates were from OPD, from the IP patients (80.56), out of that 24.14% from ICU's and rest were from wards (75.86%) table 1. Females (83.33%) were found to be more prone to Enterococcal infection as compared to males (16.67 %)

Table 1: OP and IP Distribution of Enterococcal UTI cases.

| Distribution | | No. of Isolates |
|--------------|----------------|-----------------|
| OP (19.44%) | | 7 |
| IP (80.56%) | ICU (24.14%) | 7 |
| | Wards (75.86%) | 22 |

table 2.

High prevalence of Enterococcal infection was seen in the age group 61-70 years (25%) followed by 51-60 and 71-80 (16.67%) age groups table 3.

In the present study all *Enterococci* strains showed 100% sensitivity against vancomycin and linezolid. 50% of the isolates showed resistant towards high level gentamicin. Apart from the above sensitivity pattern the urinary isolates were observed with Nitrofurantoin (88.88%), Ampicillin (55.55%), Penicillin (52.77 %) and Norfloxacin (16.66%) Fig. 1.

Discussion

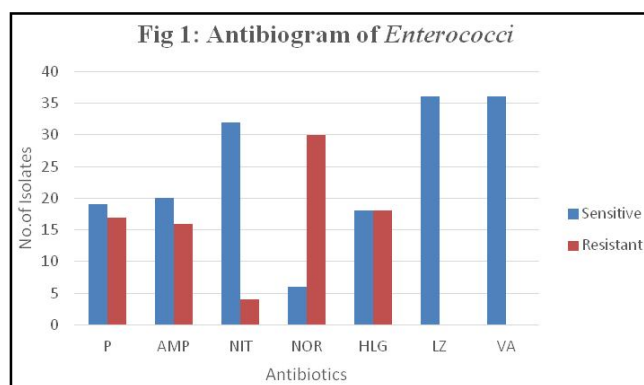
The UTI causing *Enterococci* are gaining resistance at an amplified rate to usually used antimicrobial agents. The sensitivity pattern is varying in various geographical areas (Saleh *et al.*, 2009). Regular survey of antimicrobial resistance plays a very important role in the empiric

Table 2: Sex Vs Isolation (%).

| Sex | Frequency | Percentage (%) |
|--------|-----------|----------------|
| Male | 6 | 16.67 |
| Female | 30 | 83.33 |
| Total | 36 | 100 |

Table 3: Age Distribution of *Enterococci* spp. UTI cases.

| Age | Frequency | Percentage (%) |
|-------|-----------|----------------|
| <10 | 2 | 5.56 |
| 11-20 | 3 | 8.33 |
| 21-30 | 2 | 5.56 |
| 30-40 | 3 | 8.33 |
| 41-50 | 3 | 8.33 |
| 51-60 | 6 | 16.67 |
| 61-70 | 9 | 25 |
| 71-80 | 6 | 16.67 |
| >80 | 2 | 5.56 |
| Total | 36 | 100 |



P-Penicillin, AMP- Ampicillin, NIT- Nitrofurantoin, NOR-Norfloxacin, HLG- High level Gentamicin, LZ-Linezolid, VA- Vancomycin.

treatment of Enterococcal UTI (Pieore *et al.*, 2012). The present study shows the antimicrobial susceptibility pattern of *Enterococci* isolated from UTI patients. A total of Thirty six *Enterococci* strains were isolated from clinically suspected UTI patients the study period. The results of Females (83.33%) were found to be more prone to Enterococcal infection as compared to males (16.67 %). This result is closely significant with the studies of Bharti *et al.*, (2016) and Shrivastav *et al.*, (2013). In this study, High prevalence of Enterococcal infection was seen in the age group 61-70 . The finding is coined with the results of Toshiki *et al.*, (2015) a retrospective study conducted in Japan.

The present study, antibiotic susceptibility pattern of Enterococcal isolates towards vancomycin and linezolid concurred with the observations of Bharti *et al.*, (2016) and Abdulla and Abdulla (2006).

The 50% of the isolates showed resistant towards high level gentamicin. Earlier studies in Iran have reported the prevalence of HLGR strains in clinical samples to be about 52% (Feizabadi *et al.*, 2006). The treatment of choice for serious Enterococcal infections is an aminoglycoside in combination with a cell wall active agent (Landman and Quale, 1997). However, high-level aminoglycoside resistance (HLAR) is responsible for loss of synergy between agents active on the cell wall and aminoglycosides (Vakulenko *et al.*, 2003).

The *Enterococci* isolates sensitivity pattern was observed with Nitrofurantoin (88.88%) and this finding is parallel to the studies of Ali *et al.*, (2014).

Conclusion

Enterococci have emerged from being harm less commensals to versatile lethal pathogens. Nitrofurantoin is effective in the treatment of Enterococcal UTIs. In this study shows highly sensitive towards Nitrofurantoin. These data may aid health professionals in choosing the appropriate treatment for patients with Enterococcal UTI in the region and hopefully will prevent the misuse of antibiotics.

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Ethical Approval

Ethical approval was obtained from the Institutional Human Ethics Committee, Dr. Somervell Memorial C.S.I Medical College and Hospital, Karakonam, Trivandrum, Kerala, India, on 25th of March, 2019.

References

- Abdulla, E.F. and M.E. Abdulla (2006). Antibiotic option for *Enterococcus faecalis* infections. *Pak J. Med. Sci.*, **22**: 286-90.
- Ali, S., I.A. Mirza, S. Yaqoob, A. Hussain, I. Khan and M.Y. Rafiq (2014). Antimicrobial susceptibility pattern of *Enterococcus* species isolated from patients with urinary tract infection. *Gomal J. Med. Sci.*, **12**:11-4.
- Arias, C.A., D. Panesso, D.M. McGrath, X. Qin, M.F. Mojica, C. Miller, *et al.*, (2011). Genetic basis for in vivo daptomycin resistance in *enterococci*. *N. Engl. J. Med.*, **365**: 892-900.
- Bagshaw, S.M. and K.B. Laupland (2006). Epidemiology of intensive care unit- acquired urinary tract infections. *Curr. Opin Infect Dis.*, **19**(1): 67-71.
- Baron, E.J., L.R. Peterson and S.M. Finegold (2002). Bailey and Scott's Diagnostic Microbiology. 11th ed. St. Louis: Mosby; 259-83.
- Bharti, A.K., U. Farooq, S. Singh, N. Kaur, R. Ahmed and K. Singh (2016). Incidence of Enterococcal Urinary Tract Infection and it's Sensitivity Pattern among Patients Attending Teerthanker Mahaveer Medical College and Research Centre, Moradabad, India. *Int. J. Sci. Stud.*, **3**(12): 115-119.
- Centers for Disease Control and Prevention (CDC). Nosocomial *enterococci* resistant to vancomycin - United States, 1989–1993. *MMWR Morb Mortal Wkly Rep.*, 1993; **42**(30): 597.
- Cetinkaya, Y., P. Falk and C.G. Mayhall (2000). Vancomycin-Resistant *Enterococci*. *Clin. Microbiol. Rev.*, **13**: 686-707.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 17th informational supplement, *CLSI M100-S17*. **27**(1):.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, M100S, 28thEdn. *CLSI*, **38**(1): 2018 CLSI, Wayne, PA: 19087 USA.
- Collee, J.G. and A.G. Fraser (2006). Mackie and McCartney Practical Medical Microbiology 14 Edition, 2006 Churchill Livingstone.
- Feizabadi, M.M., P. Maleknejad, A. Asgharzadeh, *et al.*, (2006). Prevalence of aminoglycoside-modifying enzymes genes among isolates of *Enterococcus faecalis* and *Enterococcus faecium* in Iran. *Microb Drug Resist.*, **12**(4): 265-268.
- Foxman, B. (2003). Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *Dis. Mon.*, **49**: 53-70.
- Foxman, B., R. Barlow, H. D'Arcy, B. Gillespie and J.D. Sobel (2000). Urinary tract infection: Self-reported incidence and associated costs. *Ann. Epidemiol.*, **10**: 509-15.
- Gordon, S., J.M. Swenson, B.C. Hill, N.E. Pigott, R.R. Facklam and R.C. Cooksey (1992). Antimicrobial susceptibility pattern of common and unusual species of *Enterococci* causing infection in the United States. Enterococcal Study Group. *J. Clin. Microbiol.*, **30**: 2373-8.
- Kaçmaz, B. and A. Aksoy (2005). Antimicrobial resistance of *Enterococci* in Turkey. *Int. J. Antimicrob. Agents*, **25**: 535-538.
- Katarzyna Hryniewicz, Katarzyna Szczypa, Agnieszka Sulikowska, Krzysztof Jankowska, Katarzyna Betlejewska and Waleria Hryniewicz (2001). Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Poland. *Journal of Antimicrobial Chemotherapy*, **47**: 773-780.
- Kolawale, A.S., O.M. Kolawale, Y.T. Kandaki-Olukemi, S.K. Babatunde, K.A. Durowade and C.F. Kplawale (2009). Prevalence of urinary tract infections among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. *Int. J. Med. Med. Sci.*, **1**(5): 163-167. *Asian J. Med. Sci.*, **3**(2): 56-60, 2011. 60.
- Kunin, C.M. (1994). Urinary tract infections in females. *Clin. Infect. Dis.*, **18**: 1-12.
- Landman, D. and J.M. Quale (1997). Management of infections due to resistant *Enterococci*: a review of therapeutic options. *J. Antimicrob. Chemother*, **40**: 161-70.
- Low, D., N. Keller, A. Barth and R. Jones (2001). "Clinical prevalence, antimicrobial susceptibility and geographic resistance patterns of *Enterococci*: results from the Sentry antimicrobial surveillance program, 1997–1999," *Clinical Infectious Diseases*, **32**(2): pp. S133–S145.
- Nisha Majeed and Ashalatha V. Nair (2017). Comparison of Bacteriological Profile & Antibiotic Susceptibility Pattern of Community Acquired & Nosocomial Urinary Tract Infection, IOSR. *Journal of Dental and Medical Sciences (IOSR-JDMS)*, **16**(6): Ver. VI (2017), 69-72.
- Pieore, R.K., M. Patrice and K. Lazare (2012). Antibiotic resistance in *E. coli* isolated from women genitalia and trend of minimal inhibiting concentration in a semi-urban population. *Current research journal of biological sciences*, **4**(16): 696-701 pp. 926-927.
- Saleh, A.A., S.S. Ahmed, M. Ahmed, A. Naser and A.M. Ruhul (2009). Changing trends in uropathogens and their antimicrobial sensitivity pattern. *Bangladesh J. Med. Microbiol.*, **3**(1): 9-12.
- Schouten, M.A., J.A.A. Hoogkamp-Korstanje, J.F.G. Meis and A. Voss (2000). Prevalence of vancomycin-resistant *Enterococci* in Europe. *Eur. J. Clin. Microbiol. Infect. Dis.*, **19**: 816-822.

- Shah, L., S. Mulla, G.P. Patel and S. Rewadiwala (2012). "Prevalence of *Enterococci* with higher resistance level in a tertiary care hospital: a matter of concern," *National Journal of Medical Research*, **2**: pp. 25–27, 2012.
- Shinde, R.S., G.V. Koppikar and S. Oommen (2012). Characterization and antimicrobial susceptibility pattern of clinical isolates of *Enterococci* at a tertiary care hospital in Mumbai, India. *Ann. Trop Med Public Health*, **5**: 85-8.
- Shrivastav, P., R. Mehta, S.P. Nirwan, M. Sharma and S.S. Dahiya (2013). Prevalence and antimicrobial susceptibility of *Enterococcus* species isolated from clinical samples in a tertiary care hospital of North India. *Natl. J. Med Res.*, **3**: 389-91.
- Tille, P.M., B.A. Forbes Bailey & amp; Scott's diagnostic microbiology Elsevier Inc. (2014).
- Toshiki Kajihara, Shigeki Nakamura, Naoki Iwanaga, Kazuhiro Oshima, Takahiro Takazono, Taiga Miyazaki, Koichi Izumikawa, Katsunori Yanagihara, Nobuoki Kohno, Shigeru Kohno (2015). Clinical characteristics and risk factors of enterococcal infections in Nagasaki, Japan: a retrospective study. *BMC Infectious Diseases*, **15**: 426.
- Vakulenko, S.B., S.M. Donabedian, A.M. Voskresenskiy, M.J. Zervos, S.A. Lerner and J.W. Chow (2003). Multiplex PCR for detection of aminoglycoside resistance genes in *Enterococci*. *Antimicrob Agents Chemother*, **47**: 1423-6.
- Wayne, P.A. (2007). Clinical and Laboratory Standards Institute, 5-11.
- Wilson, M.L. and L. Gaido (2004). Laboratory Diagnosis of Urinary Tract Infections in Adult Patients. *CID*, **38**: 1150-8.