Occurrence and antibiogram profile of multidrug resistant *E.coli* isolated from hospital waste

[*1]Warkhade B.B., and [2]Gupta S.G.

[*1]Department of Biotechnology, Badrinarayan Barwale Mahavidyalaya. Jalna.Maharashtra, India, 431213.

[2]Government Institute of Forensic Science, Aurangabad. Maharashtra, India, 431004.

ABSTRACT

The emergence of antimicrobial-resistant bacteria is a major challenges in hospital waste samples. *Escherichia coli* is one of the most public relevant agent of bacterial diseases. Indiscriminate use of antibiotics resulted in the development of multiple drug resistant *E. coli* through the world. In present study twenty one strains of antibiotic resistant *E. coli* were isolated from hospital waste samples like cotton swabs, bandages, needle etc. These isolates were identified by various morphological and biochemical test. Antibiotic susceptibility test was performed by Standard disc diffusion method. All the isolates were found to be 100% resistant to Ceftazidime (CAZ) 30mcg, Ceftriaxone (CTR) 30mcg, and Nitrofurantoin (NIT) 300 mcg.

***Key Words*:** *E. coli*, Antibiotic susceptibility, hospital waste.

INTRODUCTION

An increasing of numbers and types of infectious diseases treated in hospital and healthcare facilities has become a major challenges in safe disposal of hospital waste. About ten percent of hospital generated waste is infectious, which can be hazardous to the public (WHO, 2014). Pathogenic *E. coli* strain causes several diseases such as gastroenteritis, urinary tract infections and neonatal meningitis. So, antibiogram profile is an important study to evolve resistance pattern of *E. coli* against the utmost used antibiotics. In recent years, increase in antimicrobial resistance has become a major concern and public health issue worldwide (Jones et.al., 1999).Multidrug resistance bacterial pathogens can be accumulated in several ecological niches because of the prevalent use of commercially available antibiotics in hospital. Multiple antibiotic resistances (MARs) in bacteria may be commonly associated with the presence of plasmids (Uma et.al., 2009). *Escherichia coli* is a ubiquitous and diverse bacterium. *E.coli* is generally harmless commensal microorganism but when these bacteria acquire a transposons and virulence factors, become a pathogenic and capable of causing a variety of diseases like Urinary tract infection, gastroenteritis, and blood stream infection. The worldwide burden of these infections are staggering, with hundreds of millions of people getting affected annually.

The susceptibility patterns and characterization are of great importance as these data may be used to devise mechanisms to stem the emergence and subsequent spread of infections and drug resistance by the organism. The aim of the study was to examine antibiotic resistance patterns of *E. coli* isolated from hospital waste.

MATERIALS AND METHODS

Sample collection

The waste samples like cotton swabs, bandages, needles etc. were collected from the government hospital Aurangabad, Maharashtra, India. All the samples were collected in sterile container and brought to the laboratory.

Isolation and Identification

The collected hospital waste samples then used for the isolation purpose of *E.coli*. All the samples were enrich in the Nutrient broth for about 24Hours. Enriched culture was then transfer to the plates of Eosin Methylene Blue (EMB) agar. Representative colony types were subcultured on their isolation media until pure cultures were obtained as confirmed by microscopy. The pure cultures were tentatively characterized and identified on the basis of their colonial morphology, Gram's reaction and biochemical tests such as sugar fermentation test, methyl-red test, Vog'sproskauer test, indole test, catalase test, oxidase test, citrate agar test and oxidative fermentation test. All the isolates were maintained by glycerol stocks for further analysis.(Schofield *et al*., 2007; Uppal *et al*., 2007).

McFarland turbidity standard

The turbidity standard of the microorganisms used was 0.5. (1%) v/v solution of H_2SO_4 was prepared by adding 1 ml of concentrated H_2SO_4 to 99 ml of distilled water and mixed well. 1% w/v solution of barium chloride was also prepared by dissolving 1 g of the dehydrated salt ($BaCl_2.2H_2O$) in 100 ml of distilled water. Then 0.6 ml of the barium chloride was added to 99.4 ml of the sulphuric acid solution and was mixed well. The small quantity of the turbid solution was transferred into a test tube which was used to equate with the inoculated bacteria in Mueller Hinton broth (Cheesbrough, 2004).

The standardization of inoculums

The concentration of each of the suspension of the test organisms and the standard isolates were prepared by picking a 24 h colony of the organism using sterile wire loop into test tube containing sterile Mueller Hinton broth to form turbidity equal to 0.5 scale of McFarland's standard (1.5×10^8 cells/ml) (Coyle, 2005). The bacterial suspensions was inoculated by streaking on prepared Mueller Hinton agar with the help of sterile swab stick, then the antibiotic disc was placed on the inoculated medium aseptically with the help of sterile forceps and incubated at 37^{o}C for 24 h. The zones of inhibition created by each of the antibiotics against the test organisms and the standard strains as positive control were measured and the result was interpreted using guideline from CLSI, 2012. The results were recorded as sensitive, intermediate and resistance.

Antibiotic Susceptibility Test

The Kirby-Bauer disc diffusion method was used for Antibiotic Susceptibility test. The commercially prepared antibiotics (Himedia) used were Nitrofurantoin (NIT) 300 mcg, Ceftazidime (CAZ) 30mcg, Ceftriaxone (CTR) 30mcg, Amoxyclav (AMC) 30mcg, Cefotaxime (CTX) 30mcg, co-Trimoxazole (COT)

25mcg, Cefepime (CPM) 30mcg, Vancomycin (VA) 30mcg, Ceftazidime (CAZ) 30mcg, Nalidixic acid (NA) 30mcg, Amphicillin/Salbactum(A/S) 10/10 mcg, Ciprofloxacin (CIP) 5mcg, Cefoxitin (CX) 30mcg, Imipenem (IPM) 10mcg, Norfloxacin (NX) 10mcg, Azithromycin (AZM) 15mcg, Ofloxacin (OF) 5mcg, Piperacillin/Tazobactam (PIT) 100/10mcg, Imipenem/cilastin(I/C) 10/10mcg, Doxycycline HCl (DO) 30mcg, Gentamicin (GEN) 10mcg, Netillin (NET) 30mcg, Gatifloxacin (GAT) 5mcg, Amikacin (AK) 30mcg, Meropenem (MRP).antibiotics discs were carefully placed on the surface of Muller-Hinton agar plates seeded with purified isolate. The standardization of the bacterial suspensions was achieved using 0.5 McFarland solutions. Inhibition zone diameters were measured after 18-24 hours of incubation at 37 ^{0}C

RESULTS AND DISCUSSION

Isolation and identification of *E. coli*

The total 21 isolates of multi drug resistant E. *coli* were obtained for the hospital waste samples. Morphologically typical colonies were verified by Gram staining, IMViC tests, fermentation of sugars like glucose, lactose, xylose, sucrose and maltose, Triple sugar iron agar test, oxidase test and catalase test.

Antibiotic Susceptibility Test

The antibiotic susceptibility profile of isolates from as in Table 1. shows that *E. coli* isolates were highly resistant to Nitrofurantoin, Ceftazidime , Ceftriaxone i.e.100%; 95% were resistant to Amoxyclav, Cefotaxime, co-Trimoxazole, and Cefepime; 86% were resistant toVancomycin; 81% were resistant to Ceftazidime and Nalidixic acid; 76% were resistant to Amphicillin/Salbactum and Ciprofloxacin; 67% were resistant toPiperacidin/Tazzobactum;

Table 1. The antibiotic resistant profile of *E.coli* isolates from Hospital waste samples (N=21)

Antibiotics	Resistance (%)	Intermediate (%)	Sensitive (%)
Nitrofurantoin (NIT) 300 mcg	21(100)	0(0)	0(0)
Ceftazidime (CAZ) 30mcg	21(100)	0(0)	0(0)
Ceftriaxone (CTR) 30mcg	21(100)	0(0)	0(0)
Amoxyclav (AMC) 30mcg	20 (95)	1(5)	0(0)
Cefotaxime (CTX) 30mcg	20(95)	1(5)	0(0)
Co-Trimoxazole (COT) 25mcg	20(95)	0(0)	1(5)
Cefepime (CPM) 30mcg	20(95)	1(5)	0(0)
Vancomycin (VA) 30mcg	18(86)	3(14)	0(0)
Ceftazidime (CAZ) 30mcg	17(81)	3(14)	1(5)
Nalidixic acid (NA) 30mcg	17(81)	1(5)	3(14)
Amphicillin/Salbactum(A/S) 10/10 mcg	16(76)	4(19)	1(5)
Ciprofloxacin (CIP) 5mcg	16(76)	1(5)	4(19)
Piperacidin/Tazzobactum(PTI)100/10mcg	14(67)	3(14)	4(19)

Cefoxitin (CX) 30mcg	13(62)	1(5)	7(33)
Imipenem (IPM) 10mcg	12(57)	6(29)	3(14)
Norfloxacin (NX) 10mcg	12(57)	2(10)	7(33)
Azithromycin (AZM) 15mcg	10(48)	6(29)	5(24)
Ofloxacin (OF) 5mcg	10(48)	3(14)	8(38)
Piperacillin/Tazobactam (PIT) 100/10mcg	10(48)	0(0)	11(52)
Imipenem/cilastin(I/C) 10/10mcg	9(43)	5(24)	7(33)
Doxycycline HCl (DO) 30mcg	9(43)	1(5)	11(52)
Gentamicin (GEN) 10mcg	9(43)	2(10)	10(48)
Netillin (NET) 30mcg	7(33)	0(0)	14(67)
Gatifloxacin (GAT) 5mcg	7(33	0(0)	14(67)
Amikacin (AK) 30mcg	5(24)	1(5)	15(71)
Meropenem (MRP) 10mcg	4(19)	3(14)	14(67)

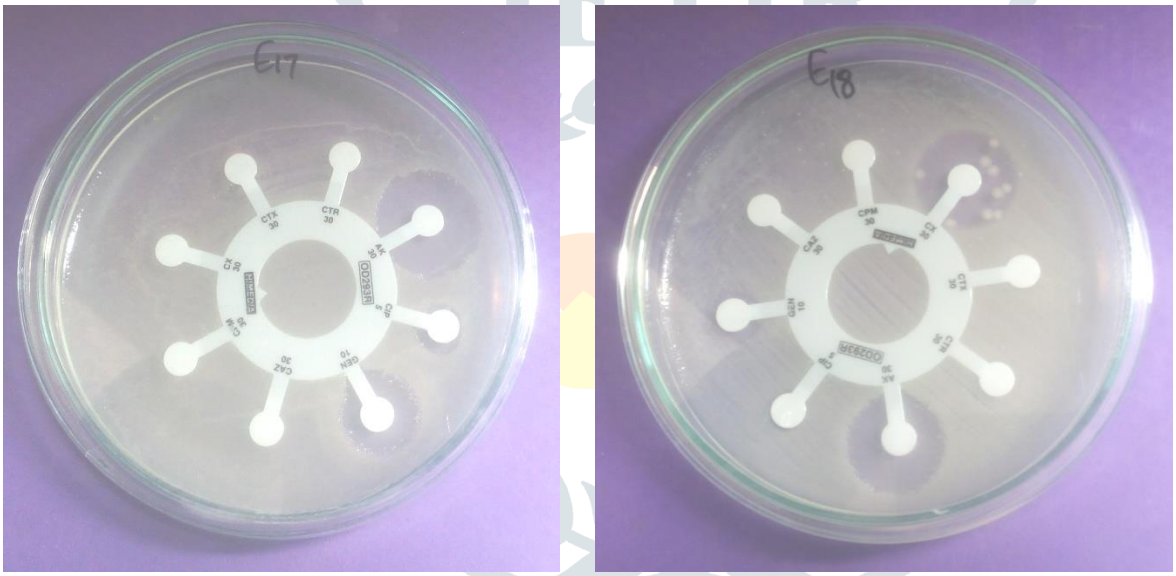

Figure 1: Octadisc Showing Multidrug Resistant *E.coli* isolates E17 and E18.

48% were resistant toAzithromycin, Ofloxacin, and Piperacillin/Tazobactam; 43% were resistant toImipenem/cilastin, Doxycycline HCl, and Gentamicin; 33% were resistant to Netillinand Gatifloxacin; 24% were resistant toAmikacin; 19% were resistant toMeropenem.

While, 71% of the isolates of *E.coli* were susceptible to Amikacin; 67% were susceptible to Netillin, Gatifloxacin, and Meropenem; 52% were susceptible to Piperacillin/Tazobactamand Doxycycline HCl; 48% were susceptible to Gentamicin; 38%were susceptible to Ofloxacin; 33% were susceptible to Norfloxacinand Imipenem/cilastin; 24% were susceptible to Azithromycin; 19%were susceptible to Ciprofloxacin and Piperacidin/Tazzobactum; 14%were susceptible to Nalidixic acid and Imipenem; 5%were susceptible to co-Trimoxazole,Ceftazidime, and Amphicillin/Salbactum Earlier study by Umolu et al., 2006 in Nigeria on pathogenic *E. coli* isolated from different hospital waste specimens showed 66% multiple drug resistance against 7 commonly used antibiotics. Drug resistances among pathogenic isolates of bacteria have been

reported earlier (Zhanel et.al., 2000). In 2000, multiple antibiotic resistance in USA was reported only 7.1 percent. Pathogenic isolates of *E. coli* have comparatively high possibilities for developing resistance (Karlowsky et.al.,2004). There are various mechanisms by which bacteria acquires resistance to antimicrobial agents such as up regulating or down regulating production of enzymes that inactivates antimicrobial agents, altering the target protein to which antimicrobial agents binds and efflux mechanisms through which bacteria expel drug from the cell (Jacoby et.al.,1991).Hospital waste contains pathogens in mass, in their invisible forms. So proper controlling is essential to maintain hygienic, aesthetics and cleanliness. If this extensive amount of waste is not properly managed it can case the pollution of air soil, and water. Again it can cause dangerous diseases, either in epidemic, endemic or sporadic forms. Proper management means proper collection, separation, storage, transport and treatment of waste in harmless manner. public awareness is needed about hospital waste hazards and by making mandatory to officials of the institutions to follow the guidelines of Supreme Court and Ministry of Environment Forest, Government of In notification for biomedical (Dwivedi et.al.,2009).

CONCLUSION

Resistance to antimicrobial agents is a problem in communities as well as health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population. Factors that could be associated with transmission of resistant strains of these microorganisms include poor attention to hygiene, overcrowding, lack of an effective infection control program, and shortage of trained infection control providers. The phenomenon of multiple resistance to antibiotics has been noticed in *E.coli* isolates in varying proportions. As hospital waste material contains these multidrug resistant bacteria, it should be handled carefully and public awareness is needed about hospital waste hazards.

REFERENCE

1. Cheesborough, M. (2004). District Laboratory manual in Tropical countries. Low price edition. Cambridge University Press, pp 36 -70.
2. Clinical and Laboratory Standards Institute (CLSI), 2012. Performance Standards for Antimicrobial Susceptibility Testing. Eighteenth Information Supplement, 28(1):34-52.
3. Coyle, M.B. (2005(. Manual of antimicrobial susceptibility testing. American Society of Microbiology press, Washinton D.C.
4. Dwivedi, A. K., & Pandey, S. (2009). Fate of hospital waste in India. *Biology and Medicine*, *1*(3), 25-32.
5. Jacoby, G. A., & Medeiros, A. A. (1991). More extended-spectrum beta-lactamases. *Antimicrobial Agents and Chemotherapy*, *35*(9), 1697.
6. Jones, R. N., Kugler, K. C., Pfaller, M. A., &Winokur, P. L. (1999). Characteristics of pathogens causing urinary tract infections in hospitals in North America: results from the SENTRY Antimicrobial Surveillance Program, 1997. *Diagnostic microbiology and infectious disease*, *35*(1), 55-63.
7. Karlowsky, J. A., Jones, M. E., Draghi, D. C., Thornsberry, C., Sahm, D. F., &Volturo, G. A. (2004). Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Annals of clinical microbiology and antimicrobials*, *3*(1), 7.
8. Schofield, C. M., Murray, C. K., Horvath, E. E., Cancio, L. C., Kim, S. H., Wolf, S. E., &Hospenthal, D. R. (2007). Correlation of culture with histopathology in fungal burn wound colonization and infection. *Burns*, *33*(3), 341-346.

9. Uma, B., Prabhakar, K., Rajendran, S., Kavitha, K., &Sarayu, Y. L. (2009). Antibiotic sensitivity and plasmid profiles of Escherichia coli isolated from pediatricdiarrhea. *Journal of global infectious diseases*, *1*(2), 107-110.
10. Umolu P, Idia, Omigie O (2006). Antimicrobial Susceptibility and Plasmid Profiles of Escherichia coli Isolates Obtained from Different Human Clinical Specimens in Lagos –Nigeria. *Am Sci*. 2(4).
11. Uppal, S. K., Ram, S., Kwatra, B., Garg, S., & Gupta, R. (2007). Comparative evaluation of surface swab and quantitative full thickness wound biopsy culture in burn patients. *Burns*, *33*(4), 460-463.
12. World Health Organization (WHO). (2014). Safe management of wastes from health-care activities. WHO Library Cataloguing-in-Publication Data. 2nd edition.
13. Zhanel, G. G., Karlowsky, J. A., Harding, G. K., Carrie, A., Mazzulli, T., Low, D. E., & Hoban, D. J. (2000). A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprim-sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin, and ciprofloxacin. *Antimicrobial agents and chemotherapy*, *44*(4), 1089-1092.