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Research Article

HOSPITAL WASTE WATER: A POTENTIAL SOURCE OF ANTIBIOTIC RESISTANT BACTERIA

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ABSTRACT

The emergence of antibiotic-resistant organisms is a major public health concern, particularly in hospitals and other health care settings. Hospitals play a major role for the emergence and spreading of antibiotic resistant bacteria. Antibiotic used in the hospital are not fully metabolized by patients and released in to the hospital waste water indicates a selection pressure on bacteria for the development of resistance. This study was carried out to investigate the presence of antibiotic resistance bacteria in hospital waste water collected from a government hospitals. Antibiotic susceptibility of the bacterial isolates was assayed according to the Kirby - Bauer disc diffusion method(NCLS,2004). The outcome of the study indicated that among the all tested antibiotics a relatively high level of resistance,55.5-66.6% for amoxicillin and33.3-87.5% for faropenem were observed.

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INTRODUCTION

The emergence of antibiotic-resistant organisms is a major public health concern, particularly in hospitals and other health care settings. Infections caused by these drug resistant bacteria are difficult to treat and therefore increase mortality and hospital cost. Hospitals are hotspots for antimicrobial-resistant bacteria (ARB) and play a major role for the emergence and spreading of antibiotic resistant bacteria. The volume of antibiotics used in hospitals exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance(Kruse et al.,1999). A fraction of administered antibiotics is not metabolized but rather is excreted from treated patients; therefore, a significant amount can be detected in hospital and municipal wastewater (Kummerer et al., 2009). Various studies have been demonstrated the presence of antibiotic and antibiotic resistant bacteria in hospital waste water (Kummerer, 2001). Ampicillin was found in concentrations of between 20 and 80 mg⁻¹ in the effluent of a large German hospital (Kummerer, 2004). Quinolones and other pharmaceuticals were also detected up to 1 g⁻¹/lit in the waste water associated with various hospitals (Thomas et al., 2007; Martins et al., 2000). and thus due to the presence of these antibiotic compounds, disinfectants metabolized drugs

from patient excrement(which potentially contain multidrug-resistant (MDR) pathogens).Hospital waste water provide an environment for the exchange of antibiotic resistance genes (ARGs) between clinical pathogens and other environmental bacteria in recipient sewers (Galvin et al., 2010; Chagas et al.,2011). The present study is an attempt to investigate the presence of antibiotic resistant bacteria in waste water associated with a government hospital of central India.

MATERIAL & METHODS

Sample collection: The water samples associated with the hospital were collected in 500 ml bottle from a government hospital of central India. 6 water sample were collected from the hospital and was usually made in early hours of the morning and transported to the laboratory within 2 hours for analysis.

Isolation of Bacteria

Serial dilutions of the sample were made in sterile normal saline solution. 0.1 ml from each dilution (10⁻³ to 10⁻⁷) was spread on the media plates. Bacterial colonies were isolated by using standard isolation technique employing spread plate method on Nutrient Agar Medium and incubated at 37 ± 1 °C for 24-48 h. based on colony morphology represented colonies were further sub-cultured and incubated for 24 h at 37 °C

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Recovered bacterial colonies were characterized on the basis of gram staining (Sherman *et al.*, 2006) and following a series of biochemical tests included tests for oxidase, methyl red, catalase activity, lactose fermentation, casein hydrolysis and sugar fermentation (Lemmen *et al.*, 2004).

Antimicrobial susceptibility testing

Antimicrobial susceptibility for each isolate was determined by the disk diffusion method. antibiotics disc with known amount of following antibiotic which were Amikacin (AK) 30 mcg, Amoxicillin/clavulanate (AMC)10 mcg, Cefaperazone (CPZ) 75 mcg, Ceftriaxone (CZX) 30 mcg, Cefuroxime (CXM) 30 mcg, Ciprofloxacin (CIP) 5 mcg, Claritromycin (CLR)15 mcg, Clindamicin (CD) 2mcg, Doxycycline (DO) 30 mcg, Faropenem (FAR)5 mcg, Gentamicin (GEN) 10 mcg, Levofloxacin (LE) 5 mcg, Linezolid (LZ) 30 mcg, Norfloxacin (NX) 10 mcg, Ofloxacin (OF) 5mcg,were placed on the surface of inoculated Mueller-Hinton (MH) agar plate. After incubation plates were observed for zone of inhibition surrounding the disc. Clear area surrounding the disc indicate that growth of organism is inhibited by the drug. Data analyses were performed using the susceptibility cut points according to the Clinical and Laboratory Standards Institute guidelines (NCCLS, 2004). Multi resistance of Gram-negative bacteria was defined as resistance to at least three antibiotics.

RESULTS

In present study different bacteria were isolated from the waste water sample associated with the hospital (n=23), Based on morphological characters, gram staining and biochemical characterization bacterial isolates were belong to following group: *Klebsiella*=6, *Pseudomonas*=8, *E.coli*= 9.The most frequently identified bacterium was *E. coli* (39.13%) followed by *Pseudomonas* sp. (34.78%) and *Klebsiella* sp. (26.08%).

The result of this study indicated that isolated bacteria in this study have become resistant to most of the tested antibiotics, and this showed that they have become multi-resistant to these therapeutic agents, thus rendering these drugs ineffective as treatments of choice for infections caused by these pathogens. This is obvious from the results of percentage of the isolates that are resistant to the tested antibiotics (Fig 1). Results showed that 23 isolates of water sample were found to be resistance (4.3-65.21%). resistance pattern of *Klebsiella* isolates showed 50 % resistance to linezolid,66.6 % resistance to amoxicillin + clavulinic acid, 55.5% resistance rate for amoxicillin +clavulinic acid, and linezolid was exhibited by *E.coli* isolates, where as low level of resistance were found against amikacin, gentamycin, levoflox, oflox and cefaperazonen. High resistance rate 87.5% and 55.5 % to faropenem was observed among *Pseudomonas* and *E.coli* isolates, low level of resistance were found against piperacillin+tazobactam, norflox and cefatrioxane. Among 23 bacterial isolates most striking drug resistant isolate which was resistant to 7 antibiotic tested was *Klebsiella* spp, followed by *E.coli* isolates to 6 antibiotics, *Pseudomonas* spp to 5 antibiotics.Among all isolates 1 isolate was susceptible to all antibiotics tested, 3(13.03) were resistant to only one antibiotics, 8(34.7) were resistant for 2 antibiotics,5(21.7) for 3 antibiotics,2(8.6) for 4 antibiotics, and 4(17.3) were resistant for 5 or more antibiotics. The overall prevalence of multiple

drug resistance (resistant to 3 or more drug) in this study was 10(43.4).

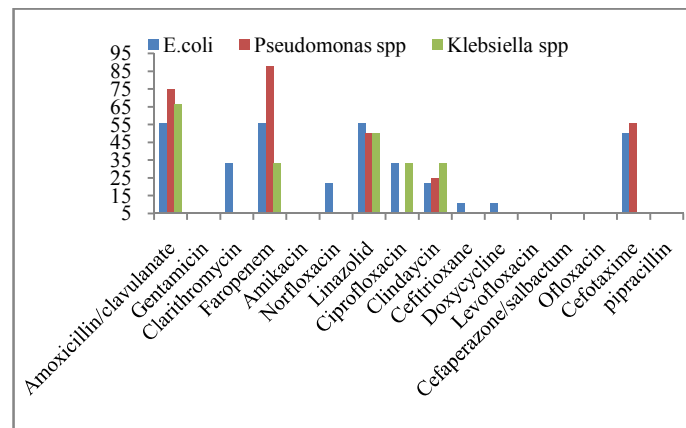


Fig 1 Percentage resistance of bacterial isolates from waste water of hospital to different antibiotics

DISCUSSION

Hospital wastewater, which receives high loads of antimicrobial agents and human pathogens, is considered a reservoir for antibiotic resistance and other genetic factors which promote the potential spread of AMR to the environment (Hocquet *et al.*, 2016). *E.coli* (39.13%) were the most frequently identified bacteria in this study, which is a member of fecal coliform bacteria and are commonly found in the intestines of animals and humans. Presence these enteric bacteria in the hospital waste water underscores the point that there is a high level of faecal contamination either directly from sewage or poor hygiene practice (Svanstrom, 2014).

Antibiotic resistance is a major public health threat, The presence of antibiotic resistant bacteria in water sources throughout the world has been documented (Kelch and Lee, 1978; Ogan and Nwiika, 1993; Young, 1993, Lien *et al.*, 2016). Indiscriminate use of antibiotics, lack of proper knowledge and negligence toward disease increase the occurrence of antibiotics resistant bacteria isolates in the hospital waste water. In Bangladesh there is clear evidence of abuse of antibiotics for which emergence of multi-drug resistant bacteria are continuously increasing (Reinthalder *et al.*, 2003). As a result, these bacteria have inevitably entered the natural environment. This is particularly true when transfer occurs in environments such as hospitals where the human population is at risk (Jamali *et al.*, 2015). Our result indicate that a moderate level of antibiotic resistance were observed in this study. overall a high level of resistance were observed against the amoxicillin. Resistance rate in *E.coli*, *Pseudomonas* and *Klebsiella* isolates for amoxicillin was found 55.5%, 75% and 66.6% respectively. This finding is consistent with the report in Bangladesh that the, resistant rate for *E.coli* and *Klebsiella* isolates were found 99% and 65% (Siddiqui *et al.*, 2015). In our study resistance rate for faropenem (belong to the penem group of antibiotics) in *E.coli*, *Pseudomonas* and *Klebsiella* spp were found to be 55.5%, 87.1% and 33.5% respectively. This was different from the other study done in Brazil where low level of resistance were observed by bacterial isolates for another antibiotics of penem group (Resende *et al.*, 2009). Amoxycillin and faropenem antibiotics are belong to the beta lactum group of antibiotics and this increase resistance is may

be due to the production of extended spectrum betalactumase, enzyme that cause resistant to penicillin such as ampicillin and amoxicillin (Brisse *et al.*, 2005; Hsu *et al.*, 2007), whereas levoflox, oflox, cefaperazone and gentamycin were found active against the bacterial isolates of waste water sample. It can be assumed that isolated bacteria in this study have been exposed to antibiotic residues. As a result mechanism of resistance to antibiotics may be developed in these isolates and persist multi drug resistance phenomena. in our study Some of the isolates resist more than three class of antibiotics and hence posses Multiple drug resistance phenomena which is an extremely serious public health problem and it has been found associated with the outbreak of major epidemic throughout the world becouse the infection caused by these multi drug resistance organism are difficult to treat. Thus, the multiple drug resistance shown by these pathogens are worrisome and of public health concern (Lateef, 2004). These bacteria, like *Pseudomonas* spp. are common environmental organisms, which act as opportunistic pathogens in clinical cases where the defense system of the patient is compromised (Lyczak *et al.*,2000).

CONCLUSION

Bacterial isolates shown a high level of resistance towards the amoxicillin. From the present investigation it can be concluded that Waste water associated with hospital contain a significant level of pathogenic and antibiotic resistant bacteria, which contribute a potential mechanism for propagation and transfer of resistant and pathogenic bacteria from hospital waste water, into the environment, which constitute an important public health concern.

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