ISOLATION AND IDENTIFICATIONS OF MULTIPLE DRUG RESISTANT BACTERIA FROM WASTE WATER OF HOSPITAL AND NON-HOSPITAL ENVIRONMENT

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ABSTRACT:

Antimicrobial agents formed the cornerstone of the treatment of infectious diseases. Resistance to antibiotics among clinical pathogens has emerged and spread progressively since the introduction of antibiotics into clinical medicine in the mid-1940s. It is estimated that, in the EU alone, antibiotic-resistant infections claim 25,000 lives every year. This study was carried out to isolate and identify public health important bacteria from waste water in hospital and non- hospital environments, and also to determine and understand the drug resistance pattern of pathogens from hospital and non hospital waste water. A total of 55 bacterial isolates were recovered from the collected samples and identified following different biochemical and morphological tests. Among the isolates, 16 (29%) were E. coli, 12 (21.3%) were Pseudomonas spp., 9 (16.4%) were Klebsiella spp., 8 (14.5%) were Salmonella spp., 5 (9%) were Staphylococcus spp., and also 5 (9%) were Vibrio spp. respectively. However, from the result of antibiotic susceptibility test, it was discovered that all isolates of E.coli, Pseudomonas, and Klebsiella and Vibrio spp. from the Hospital environment were resistant to all the antibiotics used in this study, whereas Salmonella and Vibrio spp., were found to be sensitive to some selected antibiotics.

Keywords: *Multi drug resistance Bacteria, Antimicrobial agents, waste water of hospitals, waste water of non hospital, environment.*

INTRODUCTION

Antibiotics are class of naturally occurring, semi-synthetic or chemically synthesized compounds with antimicrobial activity. They are widely used in human and veterinary medicine to treat and prevent diseases and as growth promoters in animal intensive industries. The increasing incidence of resistance to a wide range of antibiotics by microorganisms is a major concern facing modern medicine. Clinical infections, disease and death caused by resistant bacteria are increasingly common (Launay *et al.*, 2014; Chopra *et al*., 2001). Antibiotic resistant bacteria (ARB) are present and increasing in many countries worldwide causing great concern for public health (Islam 2011). Excessive use of antimicrobial agents to treat human infectious disease in both clinical settings and the general community, as well as in intensive livestock industries, has led to the increased prevalence of ARB (Wise *et al.* 1998; Mazel and Davies 1999; Islam 2011). Antibiotics place a high selective pressure on bacteria, thus eliminating the antibiotic susceptible strains and subsequently causing the proliferation and dissemination of resistant bacteria. Resistance genes can also be transferred between cells on plasmids or transposons by transductive or conjugative processes (Berger-Bachi 2002). DNA elements that mediate integration of resistance genes (e.g. integrons) may also be involved (Moura *et al.* 2012), resulting in the further spread of MDR bacteria.

There are two principal categories of ARB of concern for public health. Community-acquired (CA) strains are generally found in nonclinical settings and can affect otherwise healthy individuals. They are generally acquired via close person-to-person contact and affect mainly skin and soft tissue. Hospital-acquired (HA) strains are found in clinical settings, occur in people with predisposing risk factors and more often lead to invasive disease (Miller *et al*. 2005). HA strains are of particular concern as treatment options are limited and expensive (Tiemersma *et al*. 2004; Magiorakos *et al*. 2012). Other Possible mechanisms by which humans enhance the spread of antibiotic resistance among environmental bacteria include the deliberate or accidental introduction of antibiotics, resistant bacteria and resistance genes into the environment (Wegener *et al*., 1999; Kruse, 1999).

Hospital waste water is considered a hot spot for antibiotic resistance (AR) as a consequence of receiving a cocktail of antibiotic compounds, disinfectants, and inputs of bacterial shadings and metabolized drugs from patient excrement, which potentially contain multidrug-resistant (MDR) pathogens (Chagas *et al*., 2010, 2011; Galvin 2010). Hospital waste water can be a potential risk factor for public health and ecological balance, since it contains various hazardous components including pathogenic microorganisms (Sharpe M 2003). Moreover, owing to heavy antibiotic use, hospital waste waters contain high numbers of antibiotic resistant microorganisms than domestic wastewaters (Romero p, *et al.).* Hospital waste effluents carry pathogenic multidrug resistant microorganisms and are responsible for the spread of these organisms to the environment. Sometimes, a treated hospital wastewater can also spread multidrug resistant microorganisms (Kuhn I, *et al*.2007) Hospital waste effluents contaminate aquatic environments causing fish and other sea creatures dangerously affected. In one research, it was found that almost 80 percent of raw chickens contained multidrug-resistant bacteria and these were identical with the specimens collected from hospital patients

(Xu L, *et al.* 2011). Moreover, it has been reported that, the irrigation water system also has been contaminated by these multidrug resistant bacteria which have a chance to enter in our food chain directly. This study was carried out to isolate and identify public health important bacteria from waste water in hospital and non- hospital environments, and also to determine and understand the drug resistance pattern of pathogens from hospital and non hospital waste water.

MATERIALS AND METHODS

Study area and period

The hospital samples were collected from three different hospitals of Kano south during the period of Jan- Feb 2018. These hospitals were selected because of their importance in Kano south and their lack of wastewater treatment plant. These hospitals are located in different Local Governments of Kano south. Non Hospital waste water samples were also collected from different places of Kano south district.

Sample processing, isolation and identification of bacteria

The waste water samples were inoculated on different Bacteriological media for isolation and identification of suspected Bacterial specie. The plates were then incubated at 35 °C±2 for 24 hours. The obtained colonies were then applied for different biochemical tests. Following different biochemical and morphological characteristics, the isolates were identified.

Antimicrobial susceptibility testing

Once isolation and identification of the bacteria from collected samples were done, the standard Kirby-Bauer disk diffusion method was used to determine the antimicrobial susceptibility profiles of the isolates (Bauer, *et al.* 1996). Bacterial inocula were prepared by suspending the freshly grown bacteria in 4–5 ml sterile normal saline and the turbidity was adjusted to that of a 0.5 McFarland standard. The antimicrobial susceptibility testing was performed using Mueller-Hinton agar medium against Ampicillin (25 µg), Gentamycin (10 µg), Chloramphenicol (30 µg), Amoxicillin (30 µg), Ciprofloxacin (5 µg), Amikacin (30 µg). Kanamycin (30 µg), Penicillin (10 µg), Tetracycline (30 µg), and Vancomycin (30 µg). The plates were incubated aerobically at 37°C for 18–24 hours. The zones of inhibition were measured and compared with Clinical Laboratory Standards institute guidelines (Clinical, Institute LS. 2012).

Results and Discussion

In the present study we collected waste water samples from both hospital and non hospital environment. After processing, the isolates were identified and further analyzed for screening of multidrug resistant bacteria. A total of 55 bacterial isolates were recovered from the collected samples and identified following different biochemical and morphological tests. Among the isolates, 16 (29%) were *E. coli*, 12 (21.3%) were *Pseudomonas* spp., 9 (16.4%) were *Klebsiella spp.,* 8 (14.5%) were *Salmonella spp., 5 (9%) were Staphylococcus spp., and also 5* (9%) *were Vibrio spp.* respectively - Table (1).

From the result of antibiotic susceptibility test, it was discovered that all isolates of *E.coli, Pseudomonas, Klebsiella and Vibrio spp.* from the Hospital environment were resistant to all the antibiotics used in this study, whereas *Salmonella and Vibrio spp.,* were found to be sensitive to some selected antibiotics - Table (2). *Pseudomonas, E.coli, Salmonella and Vibrio spp.* were resistant (100%) to Ampicillin, whereas only *Staphylococcus spp.* is found to be resistant (100%) to Amoxicillin. However, samples from non hospital environments shows that *E.coli* was resistant to all antibiotics used except Gentamycin. *Klebsiella spp., Staphylococcus spp.,* and *Vibrio spp.* were found to be sensitive to Amikacin. *Salmonella spp.* and *Vibrio spp.* were sensitive to ciprofloxacin. Moreover, all the isolates from non hospital environment were resistant to amoxicillin, penicillin and Vancomycin - Table (3).

Table 1: Bacterial isolates from both hospitals and non hospital environments.

Bacterial isolates	**Hospital environment No. (%)**	**Non - Hospital environment No. (%)**	**Total No. (%)**
E.coli	10 (31.2)	6 (26.0)	16 (29)
Pseudomonas spp.	7 (21.9)	5 (21.7)	12 (21.8)
Klebsiella spp	5 (15.6)	4 (17.4)	9 (16.4)
Salmonella spp	5 (15.6)	3 (13.0)	8 (14.5)
Staphylococcus spp.	3 (9.4)	2 (8.7)	5 (9)
Vibrio spp.	2 (6.3)	3 (13.0)	5 (9)
Total	32 (100)	23 (100)	55 (100)

Table 2: Drug resistance pattern of hospital isolates

Name of the antibiotic and their disc concentration (µg/disc)	**Percentages N (%)**						
	E.coli **n=2**	***Pseudomonas spp.*** **n=4**	***Klebsiella spp.*** **n=4**	***Salmonella spp.*** **n=2**	***Staphylococcus spp.*** **n=2**	***Vibrio spp.*** **n=1**	**Total N=18**
Ampicillin (25)	5(100%)	4(100%)	3(75%)	2(100%)	1(50%)	1(100%)	15(83.3%)
Amoxicillin (30)	3(60%)	3(75%)	3(75%)	1(50%)	2(100%)	-	12(66.7%)
Amikacin (30)	4(80%)	4(100%)	2(50%)	1(50%)	2(100%)	1(100%)	14(77.8%)
Chloramphenicol (30)	2(40%)	2(50%)	2(50%)	-	1(50%)	-	7(38.9%)
Ciprofloxacin (5)	2(40%)	3(57%)	2(50%)	1(50%)	1(50%)	-	9(50%)
Gentamycin (10)	1(20%)	2(50%)	1(25%)	-	1(50%)	-	5(27.8%)
Kanamycin (30)	3(60%)	3(75%)	3(75%)	2(100%)	2(100%)	1(100%)	14(77.8%)
Penicillin (10)	4(80%)	4(100%)	3(75%)	1(50%)	1(50%)	1(100%)	14(77.8%)
Tetracycline (30)	1(20%)	3(75%)	2(50%)	1(50%)	1(50%)	1(100%)	9(50%)
Vancomycin (30)	4(80%)	3(75%)	2(50%)	1(50%)	1(50%)	-	11(61.1%)

Table 3: Drug resistance pattern of non hospital isolates

Name of the antibiotic and their disc concentration (µg/disc)	**Percentages N (%)**						
	E.coli **n=2**	***Pseudomonas spp.*** **n=4**	***Klebsiella spp.*** **n=4**	***Salmonella spp.*** **n=2**	***Staphylococcus spp.*** **n=2**	***Vibrio spp.*** **n=1**	**Total N=18**
Ampicillin (25)	2(100%)	1(50%)	3(75)	2(100)	1(50)	1(100)	15(83.3%)
Amoxicillin (30)	1(50%)	2(100%)	3(75)	1(50)	2(100)	-	12(66.7%)
Amikacin (30)	1(50%)	2(100%)	2(50)	1(50)	2(100)	1(100)	14(77.8%)
Chloramphenicol (30)	1(50%)	-	2(50)	-	1(50)	-	7(38.9%)
Ciprofloxacin (5)	2(100%)	1(50%)	2(50)	1(50)	1(50)	-	9(50%)
Gentamycin (10)	-	-	1(25)	-	1(50)	-	5(27.8%)
Kanamycin (30)	1(50%)	1(50%)	3(75%)	2(100%)	2(100%)	1(100%)	14(77.8%)
Penicillin (10)	2(100%)	1(50%)	3(75%)	1(50%)	1(50%)	1(100%)	14(77.8%)
Tetracycline (30)	1(50%)	1(50%)	2(50%)	1(50%)	1(50%)	1(100%)	9(50%)
Vancomycin (30)	1(50%)	1(50%)	2(50%)	1(50%)	1(50%)	-	11(61.1%)

Discussions

Out of the total 55 bacteria isolated from hospital and non hospital environments, 32 (58.2%) were from the hospital environments and 23 (41.8) were from non hospital environments. The rate of isolation of bacterial pathogens in the hospital environments was higher than non hospital environment; similar result was reported by Guadabassi *et al.*, those factors other than the indiscriminate use of antibiotics in human medicine, animal husbandry and agriculture may disrupt the microbial balance in favor of resistant Bacteria. In particular, wastewater from pharmaceutical plants could play a role in the selection of antibiotic resistant bacteria in sewage (Guadabassi *et al.* 1998). In this study, the most commonly isolated bacterium in all the samples was *E.coli* 16 (29%), *Pseudomonas spp.* 12 (21.8%), *Klebsiella spp.* 9 (16.4%), *Salmonella spp.* 8 (14.5%), *Staphylococcus spp.* 5 (9%) and *Vibrio spp.* 5 (9%). This result is somewhat similar to that of Ekhaise and Omavwoya.

Conclusion

The present study demonstrated that untreated hospital waste disposal could contribute to the development of antibiotic resistance in environmental organisms. The isolated bacteria are fully resistant against commonly used antibiotics like Ampicillin and Penicillin. One isolated bacterium *Pseudomonas spp.* was resistant to 9 antibiotics out of 10 antibiotics tested. Therefore, from the current research we can conclude that, there is an urgent need to raising awareness and education on medical waste issues. Proper waste management strategy is needed to ensure health and environmental safety. It is therefore, advised to all stakeholders and the health sector authorities to look after this issue seriously and take effective ways to control the spreading of resistant gene in the environment.

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