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OCCURRENCE AND ABUNDANCE OF MULTIPLE ANTIBIOTIC RESISTANCE BACTERIA IN HOSPITAL EFFLUENT WATER

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Key words *: Antibiotics resistance, Minimum Inhibition Concentration (MIC), Multiple Antibiotic Resistance (MAR)*

Abstract – The resistance among various bacterial species to different antibiotics has emerged as a cause of public health threat in all over the world at a terrifying rate. The present study was carried out to assess the antibiotics resistance of 378 bacteria strain isolated from hospital effluent water in Sri Lanka. Antibiotic-resistant bacteria were identied using 16s rRNA sequencing and the Minimum Inhibition Concentration (MIC) was determined using agar dilution method. Multiple Antibiotic Resistance (MAR) was determined using 96 well plate method in order to calculate the MAR index. Descending trend of bacterial resistance against five antibiotics; AMX (28%), AMP (21%) SUF/TRI (9%) and SDI (9%) were detected. *Bacillus* sp. (44%) was the most frequently isolated gram positive bacteria where *Staphylococcus* sp. (23%), *Micrococcus* sp. (15%), *Streptococcus* sp. (10%), *Lactobacillus* sp. (8%) and *Streptomyces* sp. (1%) showed decending order. Among the isolated gram negative bacteria *Enterobacter* sp. (31%) and *E. coli* (31%) were the dominant bacteria strains whereas *Acinetobacter* sp. (19%), *Pseudomonas aerugenosa* (8%), *Klebsiella pneumonia* (6%), *Moraxella* sp. (3%), *Aeromonas hydrophila* (1%) were recorded in low densities. MIC limits of resistant isolates against to the tested antibiotics were ranged between 60 – 660 µg/ml. 11% of bacteria was susceptible to all the tested antibiotics; 9% of bacteria were resistant for one antibiotic, 12% were for 2 antibiotics and 68% were for 5 or more antibiotics. The results of the study revealed that release of antibiotics into the environment will lead to an emerge antibiotic resistant bacteria and it compromise effectiveness of antibacterial therapy; since the infectious organisms become resistant against more antibiotics.

INTRODUCTION

Pollution by various contaminants including toxic metals, Persistent Organic Pollutants (POPs), pathogenic organisms, Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistant Genes (ARGs) are major problem in many parts of the world (Pote *et al*., 2009; Knapp *et al*., 2012; Czekalski *et al*., 2012; Brechet *et al*., 2014; Liyanage and Manage., 2016). Hospital effluents are a particular case of anthropogenic pollutants and it is a complex mixture of chemical and biological substances (Eggen *et al*., 2014; Laffite *et al*., 2016).

Use of antibiotics and spread of antibiotic resistance in clinical settings is a well-recognized problem (Brechet *et al*., 2014). The remarkable effectiveness of antibiotics have reduced mortality linked to bacterial infectious disease in only a few decades. However, now even their over and misuse has rapidly lead to a dramatic exponential increase of multidrug resistant bacteria throughout the world which caused increasing number of infectious disease (Brandt and Gardner, 2013). Waste effluents from hospitals contain high numbers of resistant bacteria and antibiotic residues which are able to inhibit the growth of susceptible bacteria (Berendonk *et al*., 2015) as antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth. Thus, resistant bacteria serve as vectors for spread of antibiotic resistance (Makky *et al*., 2013) and this enhance risk for public health through resistance genes which are transferred from environmental bacteria to human pathogens (Makky *et al*., 2013).

Bacillus sp., *Staphylococcu*s sp. and *Streptococcus* species are the frequently encountered bacteria in hospital wastewater (Farooq, S. *et al.,* 2013). *Escherichia coli*, *Pseudomonas aeruginosa* have also been reported along with varying numbers of other nosocomical pathogens such as *Klebsiella*, *Proteus*

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and *Enterobacter* species (Guzman- Blanco *et al*., 2014). Most of these bacteria have been reported to be resistant to the commonly used antibiotics which have led to outbreak of some infectious diseases in all over the world (Farooq, S. *et al*., 2013; Guzman-Blanco *et al*., 2014). Recent studies have reveled that hospital effluent could contain multidrug resistant (MDR) Enterobacteriaceae and enteric pathogens which could pose a grave problem for human health (Buhl *et al.,* 2015).

Multidrug resistance (MDR) is defined as insensitivity or resistance of a microorganism to the administered antimicrobial medicines despite earlier sensitivity to it (Yilmaz and Ozcengiz, 2016). In contrast, chromosomal resistance which is responsible for resistance to an antibiotic or an antibiotic class, acquired resistance by genetic material acquisition may be responsible for resistance against many antibiotic or antibiotic classes (Laxminarayan *et al*., 2013). This resistance is harbored by many human and animal, pathogenic and potentially pathogenic bacteria which can easily be spread by either conjugation, transformation or transduction of resistance genes which are generally located on mobile elements (plasmids, transposons, integrons) (Stokes and Gillings, 2011; Da costa *et al*., 2013).

It was calculated that the cost of treatment may increase due to increase resistance against antibiotics which triggered the use of more strong therapies against bacteria infections (WHO., 2015).

Thus, research into the occurrence, fate, effect and risks associated with the presence of antibiotic resistant bacteria in hospital effluent water and the impact on human health is needed to be address. In Sri Lankan contest there is no data concerning resistance profiles of bacteria in hospital effluents. According to authors' knowledge this is the first report on isolation and characterization of antibiotic resistance bacteria and screening of multi-drug resistance for some selected antibiotics in Sri Lanka. The results will provide reference for the risk control of antibiotic resistance bacteria in natural environment and help authorities to take action accordingly to improve treatment facilities in hospital effluent water.

MATERIALS AND METHODS

Standards and Reagents

Tetracyclines (TET) (97%), Sulfadiazine (SDI) (96%), Sulfamethoxazon (SUF) (97%), Trimethoprin (TMP) (98%), Amoxicillin (AMX) (96%), Ampicilline (AMP) (97%), Cloxacilin (CLOX) (97%), Ciprofloxacin (CIP) (96%), Gentamycin (GEN) (96%), Azythromycin (AZY) (97%), Erythromycin (ERM) (96%) and all the HPLC grade chemicals were purchased from Sigma Aldrich, USA.

Sample collection

Effluent water and sediment samples were collected from water ways in outside from hospitals. 80 sampling locations including national hospitals, teaching hospitals, general hospitals, base hospitals and divisional hospitals in different districts were selected (Table 1, Figure 1). The samples were stored in ice box which treated with salt to kept freeze condition during transportation. Samples were stored at 4^0C in the laboratory until analysis (Satoru *et al*., 2008; Liyanage and Manage, 2016).

Total Viable Counts (TVC) of antibiotic resistant bacteria in environment samples

The Total Viable Counts (TVC) were measured using the standard pour plate method using LB medium (Lauryl-Bertani (LB) medium; Tryptone, 9.1g; 4.6g, Sodium chloride, 4.6g; Yeast extract, 4.6g; agar ,13.1; per liter) (Manage *et al.,* 2009). To enumerate

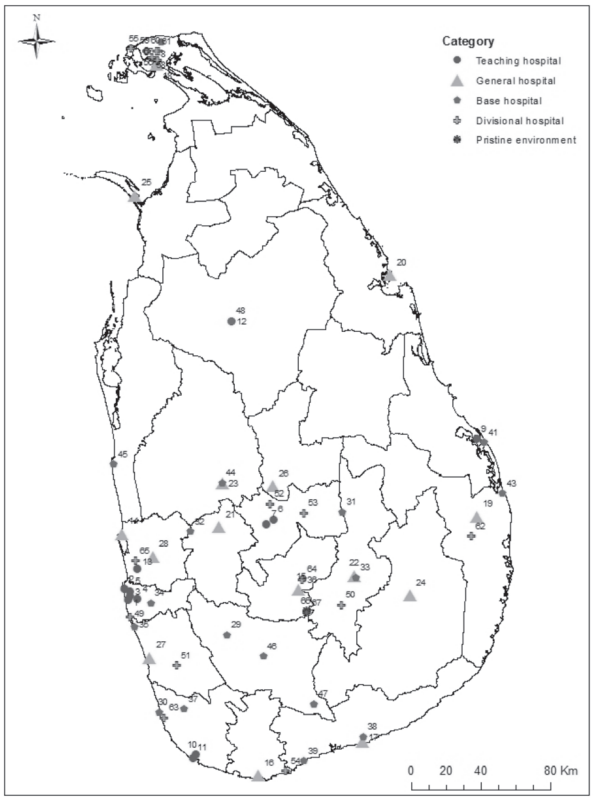

Fig. 1. Sampling locations in the present study

Table 1. Sampling locations in the present study

Hospital category		Sampling location	Hospital category		Sampling location
Teaching Hospitals	1.	Colombo Southern	Base Hospitals	42.	Anuradhapura
	2.	General		43.	Badulla
	3.	Colombo Northern		44.	Awissawella
	4.	J'pura hospital		45.	Horana
	5.	Kasal Hospital		46.	Dambulla
	6.	Kandy		47.	Point pedro
	7.	Peradeniya		48.	Kuliyapitiya
	8.	Jaffna		49.	Dehiattakandiya
	9.	Batticaloa		50.	Padaviya
	10.	Karapitya		51.	Thirukkovil
	11.	Mahamodara		52.	Medirigiriya
	12.	Anuradhapura		53.	Warakapola
General Hospitals	13.	Negombo		54.	Tissamaharama
	14.	Nuwaraeliya		55.	Muthur
	15.	Matara		56.	Kahawatta
	16.	Hambanthota		57.	Welikanda
	17.	Jaffna		58.	Moratuwa
	18.	Ampara	Divisional hospitals	59.	Kaluthra
	19.	Trincomallee		60.	Mathugama
	20.	Kegalle		61.	Akurana
	21.	Polonnaruwa		62.	Madadumbara
	22.	Gampaha		63.	Dickwella
	23.	Kaluthra		64.	Karainagar
	24.	Mannar		65.	Konadavil
	25.	Vauniya		66.	Kokuvil
	26.	Embilipitiya		67.	Manipay
	27.	Monaragala		68.	Vaddukkoddai
Base Hospitals	28.	Homagam		69.	Chunnakam
	29.	Panadura		70.	Uduvil
	30.	Ambewala		71.	Damana
	31.	Alpitiya		72.	Rathnapura
	32.	Ambalanthota		73.	Bandarawela
	33.	Tangalle		74.	Ambalangoda
	34.	Tellippalai		75.	Kekirawa
	35.	Kattankudy		76.	Lindula
	36.	Valaichchenai	Regional Hospitals	77.	Jae la
	37.	Kalmunai		78.	Asiri hospital
	38.	Kurunagala	Private Hospitals	79.	Durdans Hospital
	39.	Puttalam		80.	Nawaloka hospital
	40.	Kahawatta	Pristine environment	81.	Horton planes
	41.	Ambilipitiya		82.	Bakers fall

antibiotic resistant bacteria, filter sterilized (0.2 µm) antibiotics; TET, AMP, AMX, SUF, SDI, TMP, CLOX, CIP, GEN, AZY, ERM at final concentration of 60 µg/mL were spiked to each molting agar media (40 ^{0}C) before prepare plates (Liyanage and Manage, 2015). The colony numbers (CFU/mL) appeared on agar plates were counted after 3-5 days of incubation at 28 ^{0}C.

Isolation of antibiotic resistance bacteria

50ml of water from each sampling sites were enriched spiking with antibiotic; TET, AMP, AMX, SUF, SDI, TMP, CLOX, CIP, GEN, AZY, ERM at final concentration of 60ppm in erlenmeyer flasks. The final volume was topped up to 100ml with fliter (0.2 ìm) sterile distilled water and then the flasks were incubated at 28^{0}C ± 1^0 in 100rpm for 14 days.

At 14 days of incubation, standard pour plate method was performed to isolate and enumerate antibiotic resistant bacteria (Liyanage and Manage., 2015). Following three days of incubation at 28^{0}C, bacterial colonies with different morphological

characters were picked up and re-suspended in liquid LB medium. Subsequently pure bacterial cultures were sub cultured and stored in agar slants at -20 ^{0}C in LB-glycerol media for further studies and identification purposes.

Antibiotic susceptibility test

A loop of isolated bacterial strains were inoculated into LB broth cultures and incubated in shaking conditions (100 rpm) at 28^{0}C overnight. The cell density of the bacterial suspensions was equalized using McFarland No 0.5 (Jorgensen, J. H., & Turnidge, J. D., 2015; Liyanage and Manage, 2014). The Minimum Inhibition Concentration (MIC) was determined by using an agar dilution method following CLSI guidelines (CLSI. 2015).

DNA extraction from isolated bacteria

Isolated bacteria were cultured in 5 mL of LB liquid medium at 28^{0}C, 100 rpm for overnight. Cells were harvested by centrifugation at 12,000 rpm for 2 min, and then, the genomic DNA was extracted following kim *et al*., (2004). Purified DNA was resuspended in 30ìl of TE buffer and stored at -20 ^{0}C .

Identification of antibiotic resistance bacteria

A total volume of 200µL of gDNA product was sent to Macrogen, Korea for sequencing and DNA sequences were analyzed by the Basic Local Alignment Search Tool at the National Center for Biotechnology Information website (NCBI, http://www.ncbi.nlm.nih.gov/).

Determination of Multi-Antibiotic Resistance (MAR) bacteria

According to the CLSI (2013), Multidrug resistance was defined as resistance to two or more classes of antibiotics. Liquid bacteria cultures were prepared and equalized using McFarland No 0.5. MAR against TET, AMP, AMX, SUF, SDI, TMP, CLOX, CIP, GEN, AZY, ERM were determined at final concentration of 60 ppm of each antibiotic using 96 well plate method and absorbance were measured at 0, 12, 24 and 48 hours interval at 595nm (Naslund *et al.,* 2008). The MAR index was calculated using the following formula (CLSI., 2013).

$$\text{MAR index} = \frac{\text{Number of antibiotics which are the isolate was resistant}}{\text{Number of antibiotics which are the isolate was exposed}}$$

RESULTS

Total Viable Counts (TVC) of antibiotic resistant bacteria in environment samples

Antibiotic resistant bacteria from hospital waste water was isolated against 11 antibiotics (AMX, AMP, CLOX, CIP, TET, SUF/TRI, SDI, GEN, AZY, ERM) at final concnetration of 60 ppm. Viable (CFU/ml) count of bacteria was higher in non antibiotic supplemented control plate than antibiotic supplemented plates in all the sampling locations (Fig. 2).

The viable count range of antibiotic resistant

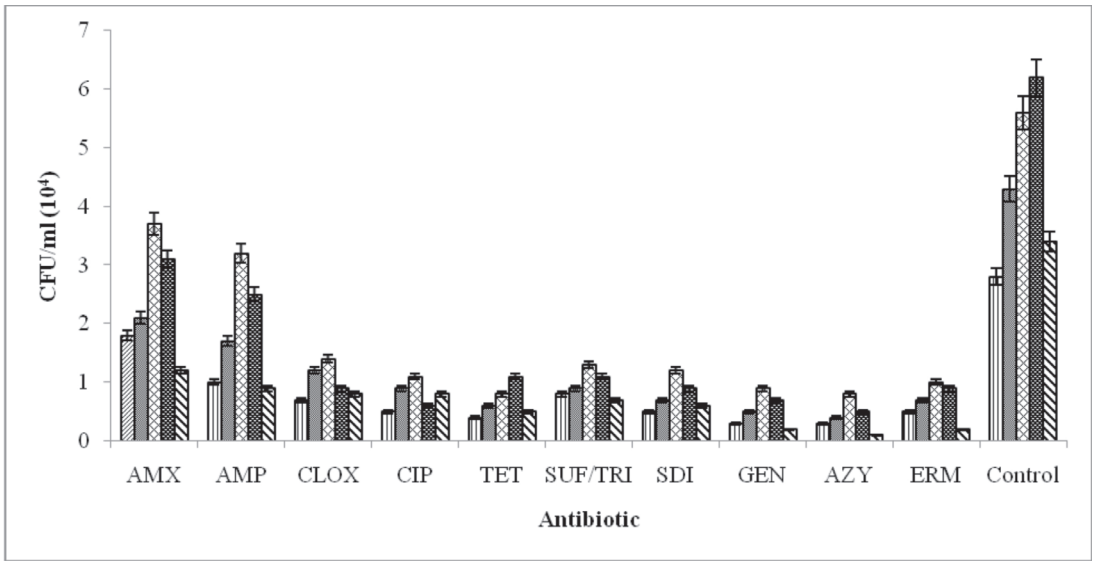

Fig. 2. Abundance of Total Viable Counts (TVC) of antibiotic resistant bacteria (▥ National Hospitals; ▩ Teaching Hospitals; ▨ General Hospital; ▩ Base Hospital; ▧ Divisional Hospital) When error bars are not shown, standard deviation was less than the width of symbol

bacteria in effluent water of National Hospitals (NH), Teaching Hospitals (TH), General Hospitals (GH), Base Hospitals (BH) and Divisional Hospitals (DH) were recorded as 0.3×10^{4}- 1.8×10^{4} CFU/ml, 0.4×10^{4}- 2.1×10^{4} CFU/ml, 0.8×10^{4}- 3.7×10^{4} CFU/ml, 0.5×10^{4}- 3.1×10^{4} CFU/ml and 0.1×10^{4}- 1.2×10^{4} CFU/mL, respectively (Fig. 2).

Maximum viable count range against AMX and AMP were recorded as 1.2×10^{4}- 3.7×10^{4} and 0.9×10^{4}- 3.2×10^{4}. Compared to the other effluent water, general hospitals samples were shown high number of resistant bacteria (0.8×10^{4}- 3.7×10^{4}) against to the tested antibioitc (Fig. 2).

The results of the study reveled that 100% of the samples were positive for one or more resistant bacteria. Among the total bacteria isolates (378), 157 (41.53%) were gram positive resistance whereas 221 (58.46%) were gram negative (Table 2 and 3). Overall bacteria isolates showed that the highest resistance against AMX (28%), following decending order AMP (21%) SUF/TRI (9%) and SDI (9%) respectively. Resistance against AMX, AMP, SUF/TRI, SDI, CLOX, TET and ERM were high (9% -28%) compared with the other tested antibiotics namely CIP, GEN and AZY (1%-2%).

The most frequently recorded gram positive bacteria was *Bacillus* sp. (44%) followed by *Staphylococcus* sp. (23%), *Micrococcus* sp. (15%), *Streptococcus* sp. (10%), *Lactobacillus* sp. (8%) and *Streptomyces* sp. (1%). Among the isolated gram negative bacteria *Enterobacter* sp. (31%) and *E. coli* (31%) showed the highest abundance and others remained relatively low abundance following *Acinetobacter* sp. (19%), *Pseudomonas aerugenosa* (8%), *Klebsiella pneumonia* (6%), *Moraxella* sp. (3%), *Aeromonas hydrophila* (1%) respectively.

Table 2. Number of gram positive bacteria isolated against to the tested antibiotics

Bacteria genera	AMX	AMP	CLOX	CIP	TET	SUF/TRI	SDI	GEN	AZY	ERM
Staphylococcus spp.* (n=35)	15	7	5	2	0	3	1	1	-	1
Streptococcus spp. ** (n=15)	6	5	-	2	-	-	-	-	-	2
Micrococcus spp. *** (n=23)	7	4	-	3	2	2	7	-	-	-
Bacillus spp. **** (n=69)	14	12	10	7	4	4	5	3	5	5
Lactobacillus spp. ***** (n=12)	4	1	-	2	3	-	3	-	-	2
Streptomyces sp. ****** (n=3)	-	2	1	-	-	-	-	-	-	-
Total (n=157)	**36**	**31**	**16**	**19**	**9**	**9**	**16**	**4**	**5**	**10**

(n= number of species)
Staphylococcus spp.* = *S. aureus, S. epidermidis, S.haemolyticus, S.warneri*
Streptococcus spp. ** = *S. pyogenes, S.bovis, S. pneumonia*
Micrococcus spp. *** = *M.luteus*
Bacillus spp. **** = *B.cereus, B.subtilis, B.thuringiensis*
Lactobacillus spp. ***** = *L. bulgaricus*
Streptomyces sp. ****** = *S.globosus*

Table 3. Number of gram negative bacteria species isolated against tested antibiotics

Bacteria genera	AMX	AMP	CLOX	CIP	TET	SUF/TRI	SDI	GEN	AZY	ERM
Acinetobacter spp. * (n=41)	15	14	-	3	3	3	-	3	-	-
Enterobacter spp. ** (n=69)	19	19	8	-	2	6	6	3	3	3
E.coli (n=68)	13	8	3	2	6	10	11	4	4	7
Moraxella spp. ***(n=7)	2	1	-	-	2	2	-	-	-	-
Klebsiella pneumonia (n=14)	6	1	1	-	-	2	-	2	2	-
Pseudomonas aerugenosa (n=17)	6	4	3	-	1	-	2	1	-	-
Haemophilus influenza (n=1)	-	-	-	-	1	-	-	-	-	-
Aeromonas hydrophila (n=4)	-	-	-		-	4	-	-	-	-
Total (n=221)	**61**	**47**	**15**	**5**	**15**	**25**	**17**	**13**	**9**	**10**

(n= number of species)
Acinetobacter spp. * = *A. baumanii, A. haemolyticus, A. calcoaceticus, A. lwoffii*
Enterobacter spp. ** = *E. aerogenes, E.cloacea, E.ludwigii, E.pyrinus, E.pulveris*
Moraxella spp. *** = *M. bovis, M.canis*

Among the isolated resistant bacteria 3 %- 67% showed MIC range between 60-180 µg/mL where 5% - 55% of isolates having MIC between 180-300 µg/mL. Only 8% - 45% showed 300-420 µg/mL of MIC range (Fig. 3).

More than 50% of the resistant bacteria showed their MIC range from 420 to 540 µg/ml for the tested antibiotics except GEN (21%), AZY (16%), CLOX (8%) and CIP (6%). GEN (39%) and AZY (45%) resistant isolates shown their MIC range between 300-420 µg/mL while CLOX had 180 to 300 µg/ml. The lowest MIC range (60-180 µg/mL) was recorded by the CIP (67%) resistant bacteria (figure 3). Compare to the tested antibiotics, the highest number of isolates showed their MIC range greater than 660 µg/mL against AMP and AMX (Fig. 3).

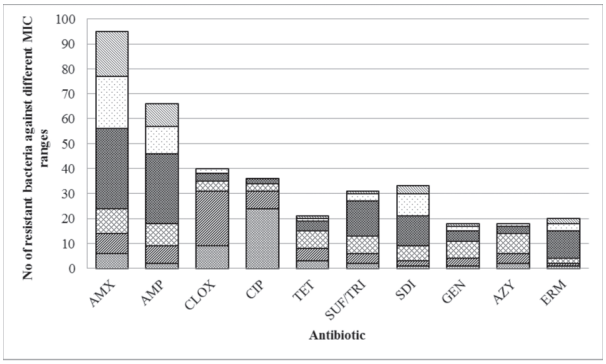

Fig. 3. MIC variations of isolated bacteria (▨ 60-180 µg/ml, ▨ 180 -300 µg/ml, ▨ 300-420 µg/ml, ▨ 420 - 540 µg/ml, ▨ 540 -660 µg/ml, ▨ >660 µg/ml)

MAR index range was varied from 0.08 to 0.51 for the isolated bacteria. It was found that MAR was highest within gram positive (MAR 0.08 -0.51) bacteria than the gram negative (MAR 0.08 -0.45) bacteria. The highest MAR index was found for *Streptomyces* sp. (0.51) and the lowest was for *Bacillus* sp. (0.08) and *Enterobacter* sp. (0.08) respectively (Fig. 4).

Among 378 resistant bacteria isolates, 40 strains were belonging to; *Staphylococcus* sp. (3), *Streptococcus* sp. (2), *Micrococcus* sp. (3), *Bacillus* sp. (8), *Lactobacillus* sp. (1), *Acinetobacter* sp. (7), *Enterobacter* sp. (9), *E.coli* (7) and they showed resistant against all the tested antibiotics. *Aeromonas hydrophila* was resistant against AMX, AMP and SUF/TRI. *Streptomyces* sp., *Moraxella* sp., *Pseudomonas* sp., *Haemophilus influenza* and *A. hydrophila* were not shown resistant against CLOX and CIP.

The overall resistance to AMX was 92%, followed by AMP (90%), SDI (68 %), SUF/TRI (65 %), TET (62 %), ERM (58 %), GEN (47 %), AZY (40 %), CLOX (16 %) and least resistant was recorded for CIP (11%) (figure 3).

DISCUSSION

Indiscriminate use of antibiotics has led to an increasing incidence of antibiotic resistance in worldwide (Aminov, 2010). At present heavy use of antibiotics in hospitals and immune compromised

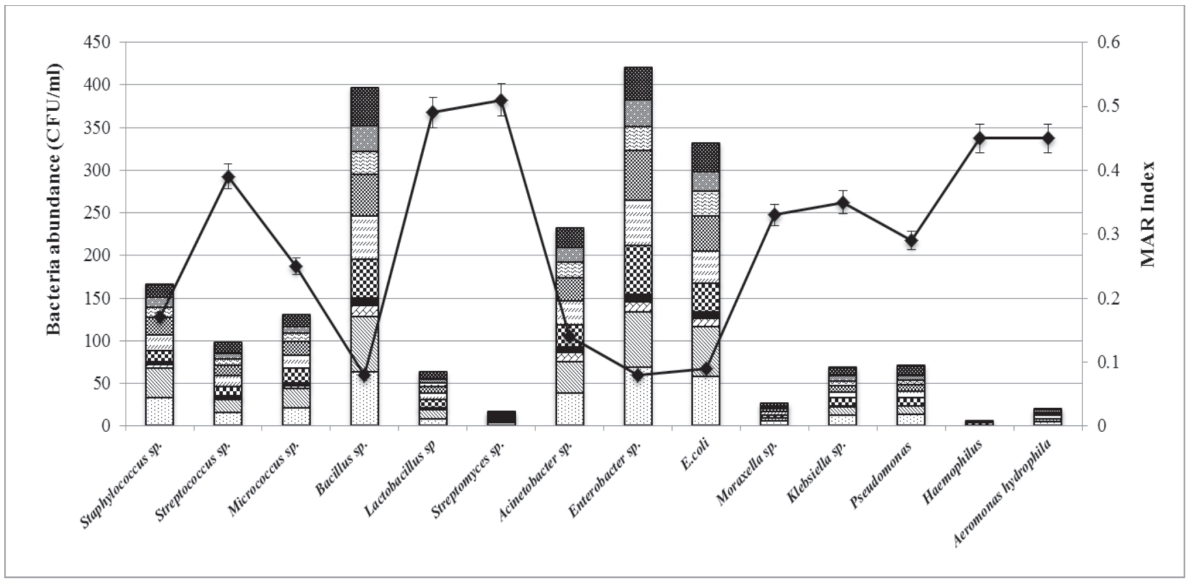

Fig. 4. Multiple drug resistance of isolated bacteria and Multiple Antibiotic Resistance (MAR) index (▨ AMX, ▨ AMP, ▨ CLOX, ■ CIP, ▨ TET, ▨ SUF/TRI, ▨ SDI, ▨ GEN, ▨ AZY, ■ ERM; Line graph indicated the MAR)

become greater concern from national and international health agencies due to development of antibiotic resistance (Aminov., 2010; WHO., 2015).

Liyanage and Manage., (2016) have shown that contaminations of AMX (0.001-0.023ppm), TET (water: 0-0.001ppm, sediments: Not detetcted), SDI (water: 0.001- 0.003ppm, sediments: 001-0.003ppm), SMX (water: 0.001- 0.018ppm, sediments: 0.001-0.002ppm) and ERM (water: 0.001-0.008ppm, sediments: 0.001-0.003ppm) in hospital effluent water in Sri Lanka. It is well known that antibiotic residues in hospital effluent water enhanced development of antibiotic resistance. The U.S. Centers for Disease Control and Prevention (CDC) estimates that antibiotic resistance is responsible for more than 2 million infections and 23 000 deaths occur each year in the U.S. at a direct cost of $20 billion (CDC., 2013). Although reliable estimates of economic losses in the developing countries have not been calculated so far due to lack of information (Laxminarayan *et al*., 2013).

In the present study it was found that high abndance of resistance bacteria (58%) against to â-lactams such as AMP, AMX and CLOX. Similarly high incidence of resistance to β-lactams (43%) as well as protein inhibiting antibiotics like tetracycline (25%) was reported by Rice in 2012.

The most recent worldwide estimates of global antibiotic resistance has revealed that *E.coli*, *K. pnumonia* and *S. aureus* are the three pathogenic bacteria that greatest concern associated with both hospital and community acquired infections (WHO., 2015). In the present study frequently identified bacteria was *Enterobacter* sp. (21%) followed by *Bacillus* sp. (21%), *E.coli* (20%), *Acinetobacter* sp. (11%), *Staphylococcus* sp. (10%), *Micrococcus* sp. (6%), *Streptococcus* sp. (4%), *Klebsiella pneumoniae* (4%), *Pseudomonas* sp. (4%), *Lactobacillus* sp. (3%), *Moraxella* sp. (2%), *Aeromonas hydrophila* (1%) and *Haemophilus* sp. (0.2%). More or less similar results on *E.coli* (18%) and *Bacillus* sp. (15%) in hospital effluent water was documented as well (Dallal *et al.,* 2010).

More than 110 of bacteria isolates showed their MIC at middle range (420 -540 µg/mL) against the tested antibiotic except GEN, AZY, CLOX, CIP. Similar trends of the antibiotic MIC levels in the members of Enterobactericiae have been reported by many of the researchers (Gelband *et al.,* 2015; Farooq, S. *et al*., 2013). It was revealed that 2.6% of bacterial isolates were resistant to GEN and CIP at concentration up to 10ppm (Santos *et al.,* 2010). Accordingly to Santos *et al.,* (2010) least active antibiotic tested was sulphamethoxazole, as 83 % of the isolates were resistant against at 25 ppm of the antibiotics while 63 % were resistant to 1020 ppm. Similarly, the resistant pattern of the bacterial isolates against ciprofloxacin (MIC= 180-420 ppm) was lower in the present study. This idea is supported by Moges *et al.* (2014) that, all urinary tract pathogens such as *E. coli, Klebsiella* spp. *Citrobacter* spp were 100% susceptible to ciprofloxacin in Brazil.

Multi drug resistance against *E.coli, Staphylococcus* sp., *Acinetobacter* sp., *Klebsilla* sp., *Pseudomonas aeruginosa*, *Haemophilus* sp. and *Aeromonas hydrophila* strains particularly significant because they are associated with GI illnesses and many other human infections. Based on the recent findings, most of the isolated *E.coli* were typically multidrug resistant bacteria and common causative agent for childhood diarrhea (Ochoa *et al*., 2009). Further it has been reported that multidrug resistance in *Klebsiella* and *Proteus* spp. were medically important among the causative agents of urinary tract infections (Moges *et al*., 2014).

Multiple antibiotic resistances are known to arise due to acquisition of resistance genes through genetic exchange, mutation and physiological mechanisms such as possession of specific proteins and efflux pumps (Davies and Davies., 2010). Thus, significant rise in drug resistant bacterial contamination exhibited by gram negative and positive bacteria including pollution indicator organisms is a risk to public health, particularly due to the emergence of resistance within microbial diversity. Antibiotic resistance bacteria tend to adopt various resistance mechanisms to survive in the unfavorable conditions. Thus, improved knowledge of molecular mechanisms controlling multidrug resistance should facilitate the development of novel therapies to combat these intransigent infections.

CONCLUSION

Bacterial resistance to antibiotics has been frequently associated to indiscriminate use of antibiotics. The presence of antibiotic resistant organism in hospital effluent water can be creating problems for public health. Since this organism may be vital to the safety and well-being of patients who are hospitalized as well as individuals who are susceptible to infection. Therefore, proper waste water treatments plants are needed for hospitals to minimize the distribution of

antibiotic resistance bacteria in the environment.

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