Survey of Hospital Drains Antibiotic Resistant Bacteria for Hygiene and Health Care

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Abstract - The antimicrobial susceptibility of twelve bacterial isolates obtained from different three hospital drains in Cairo, Egypt isolated within three months was determined, using agar diffusion method. Only twelve out of twenty-eight antibiotic resistant bacterial isolates were selected according to their sensitivity for equal or more than two antibiotics used. Isolates from hospital drains were subjected to antimicrobial susceptibility testing and the percent of resistance bacteria were subjected to thirteen antibiotics as the following: CN (58.3%), CAZ (91.7%), CTX (91.7%), TOB (83.3%), CEP (83.3%), IPM (25.0%), SXT (33.3%), VA (75.0%), AK (25.0%), SAM (58.3%), FEP (50.0%), CIP (8.3%), and CRO (75.0%). The relationship between plasmid and antibiotic resistant bacteria explained from pharmaceutical products isolates and detected two plasmids while isolates from hospital drains detected also two plasmids and one isolate detected four plasmids.

Keywords - Antibiotic resistant bacteria, Pharmaceuticals, Hospital drain.

I. Introduction

PHARMACEUTICAL products of various forms and dosage are susceptible to contamination by a variety of microorganisms during manufacturing and use. Such products are considered microbiologically unsafe, if low levels of pathogenic or higher levels of opportunistic pathogens are present or toxic microbial metabolites persist even after death or removal of all microorganisms present or detectable physical and chemical changes have occurred in the products. The use of such products, even where the level of contamination is low may present potential health hazards to patients. In addition, such spoilt products constitute wastage and may have serious economic implication for the manufacture. Orally administered drugs often contain non-pathogenic microorganisms [1][1]

During recent years, the issue of pharmaceutical compounds (PhCs) in wastewater has become a major concern in terms of both human health and the environment. This has prompted the launch of several monitoring studies into the most commonly administered compounds in urban wastewater [2]-[4] and surface water [5].

Hospital wastewaters are composed of the effluents of different services: kitchen, internal laundry, heating and cooling systems, laboratories, radiology departments, outpatients departments, transfusion centres and wards. Due to the nature and quantity of the micro-pollutants they harbor, such as active substances of medicines and their metabolites, chemicals, heavy metals, disinfectants, sterilizers, and radioactive markers, which are typically present at concentrations of µg/L, they should be earmarked for special consideration. Previous studies investigated the occurrence in hospital effluents of detergents, disinfectants, organic compounds (alcohols, acetone, formaldehyde, acetaldehyde, phenols) and several metals [6], [7] and the proliferation of drug-resistant microorganisms [8].

Antibiotic resistance has become a major clinical and public health problem within the life time of most people living today [9]. Confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem [9].

The aim of this study is focused on isolation, purification and identification of microbial contamination from different hospital drains in Egypt under study and an attempt to generate original local data and examine the possibility of contaminated hospital effluents contributing to the resistance problems.

II. Materials And Methods

A. Samples Collection and Storage

Samples of hospital drains were collected from final effluents from three hospitals (A), (B), and (C) (n=4) for each hospital in Cairo, Egypt. Samples were collected within three months in 250 ml glass bottles pre-sterilized and transported to the laboratory in a cooler and stored at 4°C in the chiller.

B. Antibiotics used against isolates from hospital drains

About thirteen antibiotic sensitivity test discs (Hi-Media Laboratories, India) with their concentrations were used to detect antibiotic sensitivity of the bacterial isolates: gentamycin (CN) (10µg), ceftazidine (CAZ) (30µg), ceftriaxone (CRO) (30µg), cefotaxime (CTX) (30µg), tobramycin (Tob) (10µg), cefoperazone (CEP) (75µg), imipenem (IPM) (10µg), cotrimoxazole (SXT) (25µg), vancomycin (VA) (30µg), amikacin (AK) (30µg), ampicillin sulbactam (SAM) (20µg), cefepime (FEP) (30µg), and ciprofloxacin (5µg) using disc diffusion method according to [10].

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C. Media Used

Three media were used to collect bacterial samples other than selective media: Peptone tween water (1gm**:** 10ml to 1000ml deionized water) in pharmaceutical drugs and peptone water (1 gm to 1000ml deionized water) in water dilutions; Tryptic soya agar for bacterial count; Muller Hinton agar for determination of antibiotic resistant bacteria; and different selective media used (Difco Laboratories): Mueller Hinton Agar pH 7.4; Vogel Johnson Agar pH 7.2; BBL™ Mannitol Salt Agar pH 7.4; Baird-Parker Agar Base; EY Tellurite Enrichment pH 6.8; Soya Casein Digest agar media pH 7.3; Bacto™ Tryptic Soy Broth; Cetrimide agar pH 7.2; *Pseudomonas* agar pH 7.2 for detection of fluorescin; *Psudomonas* agar medium pH 7.2 for detection of pyocyanin; BBL™ Lactose Broth pH 6.9; Selenite Cystine Broth pH 7.0; Tetrathionate broth base pH 8.4; Brilliant Green agar 6.9; Scharlau Xylose Lysine Deoxycholate agar pH 7.4; Bismuth Sulfite agar pH 7.5; BBL™ TSI Agar pH 7.3; MacConkey agar pH 7.1; BBL™ Eosin Methylene Blue Agar, Levine pH 7.1; Blood Base agar pH 7.0; and Brain Heart Infusion Broth.

D. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the isolates was determined by means of the agar diffusion method, according to guidelines established by National Committee for Clinical Laboratory Standards (NCCLS) [10]**.**

E. Plasmid isolation

Plasmid isolation [11] was electrophorated (using Hofer HE 99Xmax submarine electrophorasis unit**)** [11] on 0.7 agarose gel and 1X TBE buffer at consistent 85 volt for about 3hr. the different bands sizes determined against lambda ECOR1 (marker) (21.226, 7.421, 5.804, 4.878, and 3.530 kb) and separated bands were stained by 0.1 µg/ml ethidium promide and photographic using gel document unit.

III. Results

A. Isolation of bacteria from Hospital Drains

In present study, about twenty-eight bacterial isolates were obtained from the three Hospital drains under study. Twelve out of twenty-eight isolates selected and tested for antibiotic susceptibility used in this study which was 4 isolates from each Hospital drain, the results recorded in **Table (I).** The percent of resistance bacteria from hospital drains were subjected to thirteen antibiotics were as the following: CN (58.3%), CAZ (91.7%), CTX (91.7%), TOB (83.3%), CEP (83.3%), IPM (25.0%), SXT (33.3%), VA (75.0%), AK (25.0%), SAM (58.3%), FEP (50.0%), CIP (8.3%), and CRO (75.0%). All twelve antibiotics resistant bacterial isolates were selected according to their sensitivity for equal or more than three antibiotics used, and expressed with **(42.86%).**

TABLE I
ANTIBIOTIC SENSITIVITY TEST FOR BACTERIAL ISOLATES FROM HOSPITAL DRAINS

Isolate Code	Antibiotics used												
	CN	*CAZ*	*CTX*	*TOB*	*CEP*	*IPM*	*SXT*	*VA*	*AK*	*SAM*	*FEP*	*CIP*	*CRO*
1S HG+	*R*	*R*	*R*	*R*	*R*	S	ND	S	S	S	ND	ND	*R*
2S HG-	S	*R*	*R*	S	*R*	S	ND	*R*	*R*	*R*	ND	ND	*R*
3S HG-	S	*R*	*R*	*R*	*R*	S	ND	*R*	S	S	*R*	ND	*R*
8S HG-	*R*	*R*	*R*	*R*	*R*	*R*	*R*	*R*	S	*R*	*R*	ND	*R*
9S AzG-	S	*R*	*R*	ND	*R*	S	*R*	*R*	S	S	S	S	S
10S AzG+	*R*	*R*	*R*	*R*	*R*	*R*	ND	S	*R*	*R*	*R*	ND	*R*
11S AzG-	*R*	*R*	*R*	*R*	ND	S	*R*	*R*	S	S	ND	*R*	*R*
12S AzG-	S	*R*	*R*	*R*	*R*	S	ND	*R*	S	*R*	*R*	ND	ND
4S SGG-	S	*R*	*R*	*R*	*R*	S	ND	*R*	S	*R*	*R*	ND	*R*
5S SGG+	*R*	*R*	*R*	*R*	*R*	*R*	ND	S	*R*	*R*	ND	ND	*R*
6S SGG-	*R*	S	S	*R*	S	S	*R*	*R*	S	S	ND	S	S
7S SGG-	*R*	*R*	*R*	*R*	*R*	S	ND	*R*	S	*R*	*R*	ND	*R*

(*R*): Resistant; (S): Susceptible; ND: Not detected.

B. Characterization and identification of antibiotic resistant bacteria isolated from Hospital Drains.

Also, only three antibiotic resistant bacterial isolates were characterized and identified according to morphological characterization, microscopic examination and biochemical tests according to [12], the results shown in **Table (II).** From these results obtained the identification of microorganisms were suggested to be *Staphylococcus aureus* for all 1S, 5S & 10S bacterial isolates.

C. Plasmid detection of six antibiotic resistant bacteria

The antibiotic resistant bacteria isolated were examined to determine the plasmid which may be the responsible for the resistant towards antibiotics, that explaining the relationship between plasmid and antibiotic resistant bacteria, it was obvious in **Fig. 1,** and indicated that the isolates (9D), (13D) and (21D) which obtained from pharmaceutical products were detected two plasmids. On the other hand, (1S) and (5S) isolates were isolated from hospital drains detected also two plasmids, while (10S) isolate detected four plasmids.

TABLE II
MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF ANTIBIOTIC RESISTANT BACTERIAL ISOLATES FROM HOSPITAL DRAINS

Hospital Name	***(A)***	***(B)***	***(C)***
Isolate no.	**1S**	**5S**	**10S**
Morphological characteristics			
Colony shape	punctiform	punctiform	punctiform
Colony colour	gray		
Consistency		+	
Elevation	flat	flat	convex
Margin	entire	entire	entire
Microscopic examination			
Cell shape	cocci (staph)	cocci (staph)	cocci (staph)
Gram reaction	+ve	+ve	+ve
Growth on MacConkey	-	-	-
Biochemical Reactions			
Oxidase test	ND	ND	ND
Lactose	-	-	-
Catalase	+ve	+ve	+ve
Co-agulase	+ve	+ve	+ve
Heamolysis		β- heamolysis	

ND: Not detected.

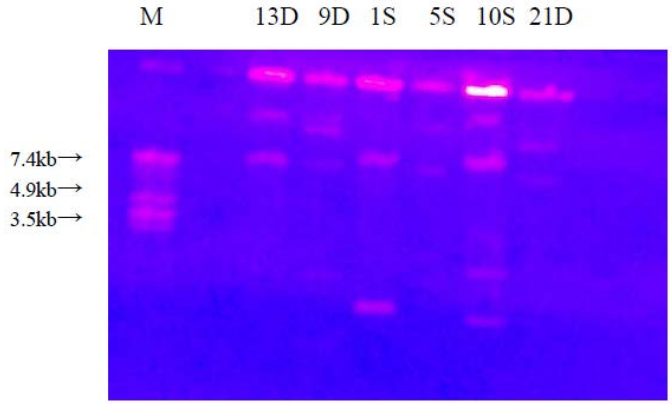

Fig. 1 Plasmid isolation by electrophoresis from six microorganisms, the lanes arranged from left to right: (M) Marker and bacterial isolate.

IV. Discussion

The aim of this study is focused on isolation, purification and identification of microbial contamination from hospital drains under study. Antibiotic have been rightly called miracle drugs but sixty years of use and measure of antibiotics has resulted in increased frequencies of resistance for most combinations of antibiotics.

Bacterial Isolates from Hospitals Drain:

The antimicrobial susceptibility of 64 strains of *S. pneumoniae* obtained from three hospitals in Porto Alegre, Brazil, isolated between 2004 and 2005, was determined, using the agar-dilution method. The prevalence of resistant (intermediate and full resistance) strains to trimethoprim/ sulphamethoxazole, penicillin, tetracycline, erythromycin, chloramphenicol, and ceftriaxone were 68%, 28%, 18%, 15%, 3%, and 1%, respectively. All strains were susceptible to vancomycin. Among 18 penicillin-resistant strains, 7 were resistant to at least two other antimicrobial drugs [13]**.**

Microbial resistance to antibiotics in clinical emerged soon after the first use of these agents in the treatment of infection disease and continues to pas significant challenge for the health care sector [14]**.** Antimicrobial resistant is intrinsically associated with the use of antimicrobial agents. In recent years antimicrobial resistance in bacteria of animal origin and it is impact on human health have drawn much attention worldwide [15]. Antibiotic resistant is a major and well-known problem in intensive care unit (ICUS) [16]**.** Gram negative bacteria of the *Enterobacteriaceae* family are important causes of urinary tract infections (UTIs), blood stream infections hospital and health care associated pneumonia and various intra abdominal infections within this family, *E. coli* is the cause of urinary tract infection, *Klebseilla pneumonia* and all *Enterobacteriaceae* have been implicated in blood stream infection and other abdominal infections.

Resistant bacteria include both pathogenic and commensal microorganisms, with the latter serving as a potential reservoir for mobile resistance elements [17]. The isolation results of antibiotic resistant bacteria from hospital drains were agreed accordance with study performed before [18] and mentioned that has hospital acquired Gram negative bacilli like *Klebsiella pneumonia* producing mutated variations of antibiotics (Cephalosporin) that made them resistant to third generation of antibiotics. He also mentioned that *E. coli, Klebsiella pneumonia, Enterobacter cloacae, Pseudomonas aeruginosa* and *Proteus mirabilis* were resistant to antibiotics, so in present study we agreed these results because some bacterial species as *Klebsiella pneumonia* and *Enterobacter cloacae* isolated from three hospitals drain, also *E. coli* isolated from Al-Zahraa hospital drain and *Pseudomonas aeruginosa* from El-Hussein hospital drain while *Proteus mirabilis* isolated from Sayed Galal drain. In contrast [19] isolated *Pseudomonas aeruginosa* strain from cystic fibrosis patients while [20], [21] were isolated *E. coli* resistant bacterial strain from different types of patients.

The isolation results of *Staphylococcus aureus* were agreed with [22], [23] and reported that Gram positive bacteria-particularly Gram positive cocci like *Staphylococcus aureus* are extremely important pathogens in hospital environment but in our study these bacterial isolates from three hospital drain in contrast results to [20], [21] were isolated *Staphylococcus aureus* resistant bacterial strains from different types of patients.

Also our results in present study was agreement to the results suggested with study [24] reported that the development of resistant through mutation can also play an important role in development of β-lactam resistance e.g. the genera *Citrobacer, Enterobacter* and *Pseudomonas*.

The study of [25] mentioned that Enterococci were the second to third most important bacterial genus in hospital infections especially *Enterococcus faecium* possesses abroad spectrum of nature and acquired antibiotic resistant but in our study Enterococci not isolated**.**

[26] investigated that the antibiotic resistant *E. coli* (87%) followed by *Klebsiella pneumonia* (10%) and others such as *Enterococcus spp*. (0.5%) and *Proteus mirabilis* (0.5%) but in our study the percentage of the antibiotic resistance bacteria such as *Klebsiella pneumonia* (25%) followed by *E. coli* and *Proteus mirabilis* (8.3%).

The present study was agreement the results suggested with study of [27],[28] who mentions that *Pseudomonas aeruginosa, Acinetobacter baumanii* and *Klebsiella pneumonia* were among the bacteria that readily developed multiple resistant mechanisms to various classes of antibiotics and also agreement with study of [29], [30] who mentioned that the rate of meat as an important vector for the transfer of antibiotic resistant from animals to human and antimicrobial resistance has always been major concern for nosocomial infections in hospital environments, such transfer can occur in three ways: by means of antibiotic resistant in food through the transfer resistance food born pathogenic or through the ingestion of resistance parts of the original food micro-flora and resistance transfer to pathogenic microorganisms.

Also agreement with results obtained by [31] who mentioned that Gram positive bacteria such as *Staphylococci* and *Streptococci* have historically and still remain major causes of human morbidity and mortality through the world because in our study three isolates *Staphylococci aureus* were resistant to many antibiotics from different three hospitals drain. There are varieties of *Staphylococcus* diseases, for example minor skin pustules, respiratory infections and sepsis.

This explaining the relationship between plasmid and antibiotic resistant bacteria *Alcalignes xylosoxidans* (9D) was detected two plasmids, *Staphylococcus xylosus* (13D) detected two plasmids and also *Staphylococcus sciuri* (15D) detected two plasmids while *Staphylococcus aureus* (1S) isolate was detected four plasmids and *Staphylococcus aureus* (5S) was detected two plasmids *Staphylococcus aureus* (10S) was detected also two plasmids.

V. Conclusion

Twelve isolates out of twenty-eight were selected from three hospital drains (four isolates from each hospital drain) in Cairo, Egypt tested through thirteen different antibiotics and the sensitivity of antibiotic resistant bacteria was expressed as 42.86%. Only three out of twelve isolates were identified as *Staphylococcus aureus* and the relationship between the plasmids and these isolates exhibited that two isolates detected two plasmids and one detected four plasmids are responsible for the resistant toward antibiotics.

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