Bacterial Contaminating the Haemodialysis Dialysate-Water Exhaust–Origin and Fate at Al-Madinah Al-Mounwwarah

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ABSTRACT: Qualitative and quantitative bacteriological analysis of the dialysate-water exhaust for the only two haemodialysis units found at Al-Madinah Al-Mounwwarah, were studied. Ten samples were collected over 75 days, 5 times from each site of sampling on biweekly basis. Enumeration of total viable (TVB) and total coliform (TC) bacterial counts CFU/ ml, showed figures ranged from 0.0 to $5231x10^2$ CFU /ml and from 0.0 to $73x10^2$ CFU /ml, respectively. Specific identification of the isolated bacterial strains using API 20 E strips confirmed that isolated bacterial populations composed of 6 genera in one unit and 7 in the other. Bacterial diversity was relatively poor, but the counts were obviously-high.Determination of $MIC_{(s)}$, $MBC_{(s)}$,as well as, the MIC/MBC indexes for 153 representative strains of the bacterial population isolated in the study were done. All the studied strains exhibited resistance to at least 3, out of the 9 tested antibiotics up to $MIC_{(S)} > 100$ µg/ml.
[Atef M. Diab and Idriss Mouneer Al-Turk. **Bacterial Contaminating the Haemodialysis Dialysate-Water Exhaust–Origin and Fate at Al-Madinah Al-Mounwwarah.** *Life Sci J* 2013;10(1):1030-1035] (ISSN:1097-8135). http://www.lifesciencesite.com. 159
Key Words: Haemodialysis, Gram–ve Bacteria of Dialysate Exhaust, Sheering effect, MIC/MBC index

1. Inroduction

The over increasing rates of renal failure and disorders is a real threat. Haemodialysis units are very special sectors in any medical center. The critical health situation of the patients being reliable for bacterial infections more than other patients because of their compromised immunity situation which originated from both the nature of the given medications and the shearing effect during dialysis scions. Although all the haemodialysis units are accompanied by special water treatment facilities in order to bring about the purest appropriate water quality that covers the clinical needs for haemodialysis.In many cases these facilities are pen pointed as a source of pollution with bacteria and their pyrogens due to working malfunctions. High counts of *Staphylococcus aureus, Streptococcus haemolyticus, Escherichia coli, Proteus mirabilis, Klebseilla pneumonia* and *Pseudomonas aeruginosa* were documented by Khedr et al., (1995), and attributed to poor quality of feed in water. So the feed in water and the concentrated dialysate solution bacterial contaminants comprise a serious health hazard on patients and can be easily detected in the final wastewater.

Wastewater released from haemodialysis units usually loaded with pathogenic, as well as, opportunistic bacteria. Untreated and/or improperly-treated of such effluents is not safe for reuse or even for discharge into natural water sources, Blumenthal et al.,(2001); Chitnis et al.,(2004). Enterobacteria such as *Enterobacter sp.*, and other Gram negative bacteria such as the non-glucose fermentors, for example *P. aeruginosa*, may persist for long periods in the environment, Chaudhry et al.,(1993); Frederico de Meirelles-Pereira et al.,(2005). Now, this massive uncontrolled re-use of wastewater, from hospitals in particular, was and still one of major threatens that man enrolled in his "Agenda 21" UNIDO(1994). Cases of catheter-related bacteremia with *Pseudomonas sp*. other than *P. aeruginosa* and *Stenotrophomonas sp*. Which were commonly reported, Capdevila et al., (1993) may add catheters as a third source of contamination. Pyrogens and dead bacterial cells comprise a corner stone in the dramatic loss of immunity in such patients, which known as sheering effect, Oettinger et al.,(1994); Lau et al., (2004). Consecutive Gram negative pathogens; *Escherichia, Serratia, Klebseilla, Pseudomonas and Proteus* (amongst 250 isolates from haemodialysis units) not only resist most antibiotics but also, showed high MIC`s, 30 - > 100 µg/ml, Winokur et al.,(2000); Baucheron et al.,(2004).

The present study aimed at surveying quantitatively and qualitatively the bacteriological pollution conditions of the dialysate-water exhaust; which simply reflect the pollution starting from the origin; the specially-treated water for haemodialysis and the dialysis concentrate.

2. MATERIALS AND MTHOD:

2.1.Materials:

2.1.1. Samples:

Samples were collected from outlets of King Fahd (Governmental hospital) and Saudi German (Private hospital) which are the only two haemodialysis units at Al-Madinah Al-Mounwwarah.

Sterilized, screw-capped, pyrogens-free glass 250 ml conical flasks, were used to collect the final dialysate

exhaust samples. Transportation regime to the laboratory within 2 hours in ice jackets with ice bags was followed. The two units were visited five times and 10 samples were collected over 75 days, 5 times from each site of sampling on biweekly basis.

2.1.2. Antibiotics:

Nine antibiotics were used o perform the sensitivity test namely Bacitracin, chloramephincol, erythromycin, impenim, penicillin G, rifampicin, streptomycin, tetracycline and vancomycin, .They are comprising the most widely- spread and commonly prescribed by physicians. They were also chosen as to represent different antibiotic families.

2.1.3.Kits:

API 20E strips kits for Gram negative Web. computer program identification (BioMerieux, Inc).

2.2.Methods:

Scheme of the work:

Continuous shaking in an orbital shaker for 3 min. at 150 rpm proceeded just before serial dilutions up to 10^{-4} were achieved.

2**.2.1.** ***Standard plate count method*****:**

Standard plate count method on plate count agar medium (Scharlau) was then adopted, inoculating 1 ml of each dilution onto 9 cm UV-sterilized plastic Petri dish, for total viable bacterial counts.

Running of a parallel set using MacConkey`s agar medium (Scharlau) was achieved for total coliform counts.Colony-forming units (CFU/ml) were enumerated using electric counter after incubation of the inoculated plates at 37º C for 24 and 48 hours, APHA (1992) .

2**.2.2.*Senstivity test*:**

Serial dilutions; 10, 30, 50, 70 and 100 µg/ml, were prepared from 9 antibiotics used.

Sterile saline solution, (0.85 % NaCl) 5 ml aliquots were inoculated with 200 µl of bacterial suspensions adjusted to a density of approx. 10^{7} to 10^{8} using basic 0.5 McFarland standard solution, NCCLS, (1990). Readings of the MIC (s) were recorded after 24 & 48 hours of incubation at 37º C. Three replication of nutrient agar plates were inoculated with 100 µl each from the tubes of MIC test starting from one tube before the MIC one, for the isolates showed resistance.

2**.2.3.*Bacterial identification*** :

It was done based on

- *Colony morphology* on nutrient agar culture medium (Oxoid),
- *Bacterioscopy of Gram-stained* bacterial smears.
- *Motility*,
- *Biochemical tests*: Standard oxidation, oxidase production, glucose fermentation, urease activity, H_2S production (from sulfur-containing amino acids), indole from tryptophan, use of citrate and decarboxylation of lysine, arginine and ornithine. Bacterial inocula from purified 24 hours freshly-cultured single colonies were adjusted to a growth density of approx. 10^{7} to 10^{8} using basic 0.5 McFarland standard solution NCCLS, (1990).

-*API 20E strips*:

API protocol adopted from the science advisory board, helped in identifying the bacterial isolates (153) up to a 95% confidence ID results.

3. Results & Discussion:

King Fahd and Saudi German haemodialysis units exhausted dialysate water showed TVB and TC counts ranged from 0.0 to $5231x10^2$ CFU/ml and 0.0 to $73x10^2$ CFU/ml, respectively. In Japan, out of 40 dialysate samples analyzed; 42.5% showed a bacterial count of more than 2000 CFU/mL, which was above the Association for the Advancement of Medical Instrumentation (AAMI) standard. Gram negative bacterial counts up to $4263x10^2$ CFU/ml was recorded, while the Gram positive did not exceed 1.8 x 10^2 CFU/ml, for both units (Fig.1, 2).

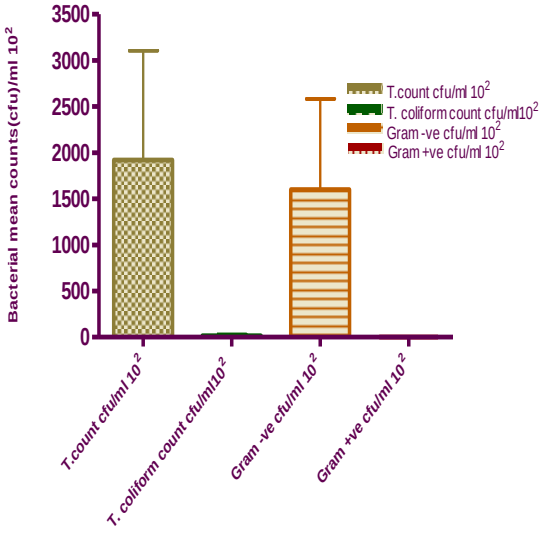

Fig. 1. *: Total viable bacteria (TVB), total coliform (TC) , Gram -ve and Gram +ve mean counts x10^2 (CFU)/ml, the dialyzate-water exhaust from the haemodialysis unit of the King Fahd hospital*

The overall distribution of isolated bacteria in the whole study showed a superior dominance of Gram negative rods (70%), then Gram positive rods (27%), Gram positive Cocci (2%) and Gram negative Cocci (1%), (Fig. 3). This was in line with what Morin (2002) concluded. The distribution pattern showed the participation of 6 species with different levels in the wastewater from King Fahd unit, where *Citrobacter frundii* .

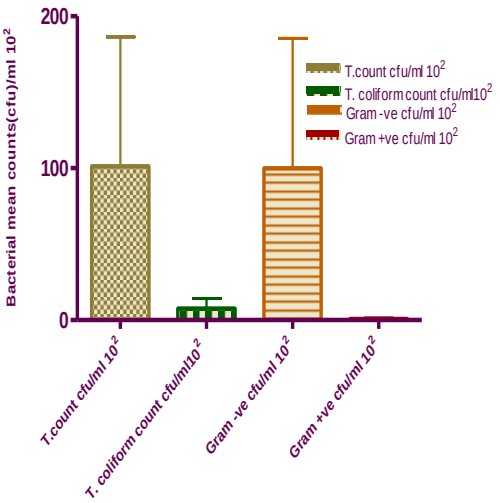

Fig. 2. *Total viable bacteria (TVB), total coliform (TC) , Gram -ve and Gram +ve mean counts x10^2 (CFU)/ml, in the final mixed wastewater from the haemodialysis unit of the Saudi-German hospital.*

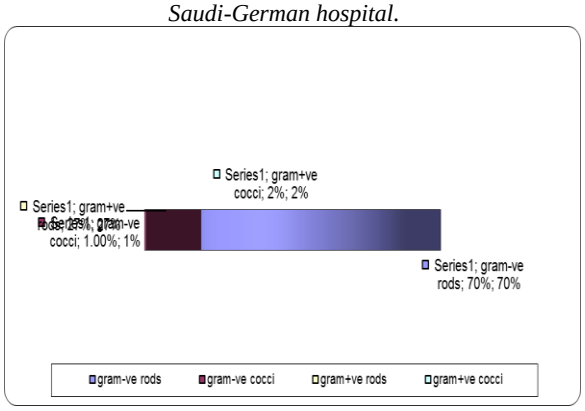

Fig. 3. : *The overall distribution of bacteria isolated from the studied haemodialysis units in this study, as gram +ve and -ve rods and cocci* took the league, followed by *Enterobacter cloacae, Enterobacter gergoviae, Chryseobacterium meningiosepticum, Chryseomonas luteda and Ochrobacterium antrhropi* (Fig.4).

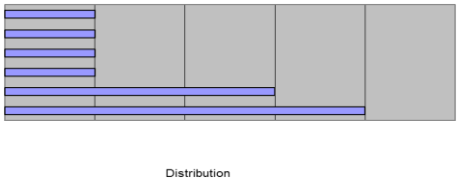

Fig. 4. : *Distribution pattern of the bacterial species isolated from King Fahd haemodialysis unit during the study period.*

The seven recorded species in the wastewater from the Saudi German unit namely; *Pseudomonas aeruginosa, Pseudomonas putida, Providencia alcalifaciens, Flavimonas oryzihabitans, Pasteurella maltocida, Chryseomonas luteda and Ochrobacterium anthropi*, were equally distributed (Fig. 5).

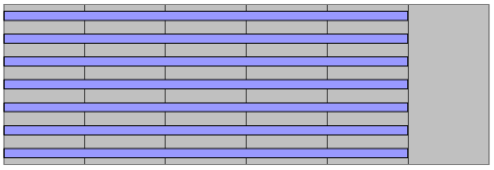

Fig. 5: *Distribution ratio of the bacterial species isolated from Saudi German haemodialysis unit during the study period.*

It seemed that members of F. *Enterobacteriaceae* dominated at King Fahd unit wastewater, while F. *Pseudomonadaceae* was the most spreading and inhabiting in Saudi German unit wastewater. All patients were dialysed for 5 h. *Pseudomonas aeruginosa* was regularly isolated in numbers up to 10^7 CFU ml^{-1} from samples of the dialysate inflow, the dialysate site and the dialysate outflow. The impermeability of the dialyzer membrane for bacteria**,** may explain low pyrogenicity of *P. aeruginosa*. Bacteriological monitoring program is a must to control and prevent haemodialysis-associated syndromes. Because dialysis machines are susceptible to microbial contamination, it is necessary to take measures such as placing an ultrafiltration membrane into the circuit before the entrance of dialysate into the dialyser, Oie et al.,(2003); Tang et al.,(2011).A diverse bacterial community was detected, and ecological and clinical consequences are discussed and found the genera *Pseudomonas*, *Sphingomonas Acinetobacter*, *Mycobacterium* and *Brevibacterium* in the build-up of biofilms, represented a health risk to patients under haemodialysis treatment.. Inspection of the dialyzer machines revealed that air-traps and heater-unit for the incoming (untreated) tap water before mixing with the dialysate concentrate were the only sites where high bacterial release was feasible, as this part of the machine escaped disinfection due to the construction of these devices, Watzke et al., (2004).

The resistibility profiles of all the identified isolates (153) against 9 antibiotics were determined and recorded in Table (1). Results were almost clear after 24 hours, but some bacterial isolates showed slow and scanty growth after 48 hours. This may be because of the extreme nature of ecosystem they isolated from. This, of course, would take a little more time of relative recovery under the laboratory conditions. One way or another, many other authors adopted the same interpretation, Putman et al.,(2000); Grkovic et al.,(2002); Merz et al.,(2004)**.** The majority of these

strains were resistant to at least 3 antibiotics with MIC (s) ranged from 50 to 100 µg/ml. Resisting all of the 9 examined antibiotics was recorded for some species to more than 100 µg/ml, Table (1). It is important here to declare that 100% of the examined strains belonged to F. *Enterobacteriaceae* were resistant to the 9 antibiotics. In a similar study the highest resistance rates were found in 767 *E. coli* isolates tested against 24 different antibiotics Oie et al.,(2003).Results of MIC/MBC indexes revealed that 87% of the isolates were negatively-affected with antibiotics. This may explain the irresponsiveness and/or the elevated rate of disease recurrence in the last ten years , Brooun et al.,(2000); Lepper et al.,(2002); Guan et al.,(2010); Hozzein and Goodfellow (2011).The inhibited or attenuated treated bacterium with such insufficient antibiotic doses give the bacterium the chance of recovery just after the concentration of the antibiotic in the body is getting lower than its MIC limits, Miller et al.,(2004); Wiuff et al.,(2005); Guan et al.,(2010); Hozzein and Goodfellow (201 Although both units obviously considered source of environmental pollution, King Fahd comprises the threatening one as the counts of bacteria coming out of it are hundred folds more than the permissible limits according to the (AAMI). Moreover, nearly all the bacterial load was gram-negative, known with their high virulence, pathogenicity and antibiotic resistance capabilities, Patel et al.,(2000**);** Zhi et al.,(2007); Tang et al.,(2011**).**When growing bacteria are exposed to MIC of antibiotic**s**, the sensitivity of the bacteria to the antibiotic commonly decreases with time, and substantial fractions of the bacteria survive. Using *Escherichia coli* CAB1 and antibiotic**s** of five different classes (ampicillin,ciprofloxacin,rifampin, streptomycin, and tetracycline), Wiuff *et al*. (2005) examined the details of this phenomenon and with the aid of mathematical models, developed and explored the properties and predictions of three hypotheses that can account for this phenomenon:

(i) Antibiotic decay,
(ii) (ii) Inherited resistance
(iii) (iii) Phenotypic tolerance.

Table 1: Eleven representatives of all of the isolated and identified bacterial species during the study period, where (-) sensitive to down to 10 µg/ml, (+) resistant to up to 50 µg/ml, (++) up to 70 µg/ml and (+++) resistant to more than 100 µg/ml

No.	Bacterial species (one isolate number as an example of the species)	Bacitra.	Chlora.	Erythro.	Imipenem	Penici. G.	Rifam.	Strepto.	Tetracy.	Vanco.
1	***Entero. Cloacae* (51)**	+++	+	+++	+++	+++	++	+	+	+++
2	***Entero. Gergoviae* (55)**	+++	+	+	+++	+++	-	+++	-	+++
3	***Citro. freundii* (52)**	+++	+	+	+++	+++	+++	+++	+++	+++
4	***Pseudomonas putida* (3)**	+++	+	+	++	+++	++	++	++	+++
5	***Pseudomonas aeruginosa* (1)**	+++	++	-	++	+++	-	++	-	+++
6	***Flavimonas oryzihabitans* (105)**	+++	++	-	++	+++	-	+++	-	-
7	***Chryseomonas luteda* (89)**	+++	+++	+++	+++	+++	+++	+++	+++	+++
8	***Pasteurella multocida* (82)**	+++	+	+	-	++	-	-	-	-
9	***Chryseo.. meningioseptecum* (66)**	+++	+	+++	+++	+++	+++	+++	+++	+++
10	***Providencia alcalfaciens* (33)**	+++	-	-	++	+	-	++	++	-
11	***Ochrobacterium anthropi* (122)**	+++	-	-	-	-	+	-	-	+++

It is critically urgent to proceed studies like this which run by Lepper *et al*. (2002), as they monitored the consumption of ß-lactam and other antibiotic**s** with known activity against *Pseudomonas aeruginosa* in a 600-bed community hospital, during a 3-year period from 1997 to 2000. It is generally assumed that the antibiotic prescription policy of a hospital has a significant impact on bacterial resistance rates; however, few studies and very little data are available to support this concept with valid statistical data.

The study results proved the urgent need for intensive and regular similar studies, innovation of new and careful maintenance of wastewater treatment system, regulations and acts on obligate treatments before introducing of such effluents to the environment and a very close

monitoring for hospitals to make sure that they are abide with the regulations and acts.

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12/21/2012