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Original Article

Bacteriology of the burn wound at the Bai Jerbai Wadia hospital for children, Mumbai, India-A 21 year study of predominant Pseudomonas species

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Abstract: This study aims to assess the change in the antibiotic sensitivity pattern of Pseudomonas species with time. Microbiological data of 2399 patients admitted to the burns unit of the Bai Jerbai Wadia Hospital for Children, Mumbai over a period of 21 years (1994-2014) was reviewed. The age group of patients admitted to our facility ranged from one month to fifteen years. A total of 11,402 burn wound swabs were cultured and 17507 isolates were tested for their antibiotic sensitivity. Pseudomonas was found to be 31.8% of the total number of isolates found on the burn wound which is second in line to Klebsiella species at our unit. It was found that the sensitivity of Pseudomonas species to various antibiotics tested has been restricted to very few antibiotics. The organism out plays most of the antibiotics that it is subjected to in vitro. Our efforts should now be channelized towards limiting the use of antibiotics. We must focus on preparing proper antibiotic policy which exercises control of irrelevant and excessive use of antibiotics. It should also be noted that every treatment facility has microorganisms unique to it and these change with time. It is, therefore of paramount importance to have an in-depth knowledge of the resident organisms and their antibiotic sensitivity. This will not only help to control infection related morbidity and mortality but will also curb the growing resistance to antibiotics.

Keywords: Pseudomonas, antibiotics, resistance

Introduction

Serious infections caused by P.aeroginosa remain a common complication in thermally injured patients contributing substantially to burn morbidity and mortality. It is an opportunistic Gram negative pathogen which produces many exoproducts including elastase, alkaline protease and hemolysin along with lipopolysaccharide which mediates much of its virulence. It has a flagellum, rendering it motile. Motility is critical in terms of invasion into tissue beneath a surface wound. Pruitt et al showed that in their animal models, invasive infection with high mortality followed surface seeding of a burn wound with a motile strain of Pseudomonas; while seeding it with a non motile mutant strain resulted in a significantly lower rate of wound infection and mortality [1]. The production of alginate and other cell surface enzymes including penicillinases makes the organism very difficult to treat or eradicate and predisposes the burn wound and medical equipment and devices in burn units to Pseudomonas infection and contamination.

Lawrence, in his seminal treatise spanning 50 years states that though Pseudomonas attracted attention, it was not widely recognized as pathogenic. Some clinicians favored it because its proteolytic activity helped remove slough. Its blue-green pigment, pyocyanin, was antibacterial and considered a potential antibiotic. Subsequent research showed that pyocyanin was toxic to skin epithelial cells [2]. The development of antibiotic resistance, especially amino glycoside resistance, is plasmid mediated. Transmission of amino glycoside resistance from one species of Pseudomonas to another and more importantly to other Gram negative

Table 1. Antibiotic sensitivity (%) of Penicillins & Carbapenems

YEAR	1994 to 1999	1997 to 1999	2000 to 2002	2003 to 2005	2006 to 2008	2009 to 2011	2012 to 2014	Average
Total Isolates	709	1231	1197	935	603	677	210	
Antibiotics	%	%	%	%	%	%	%	%
Penicillin	3.4	2.5	NT	NT	NT	NT	NT	3.0
Ampicillin	2.6	2.5	3.7	1	1.3	NT	NT	2.2
Cloxacillin	5.7	2.4	0.9	0.3	0.5	NT	NT	2.0
Carbenicillin	12.1	16.9	24.6	2.9	NT	NT	NT	14.1
Piperacillin	27.3	29.1	64.9	37.4	50.1	40	NT	41.5
Ticarcillin	24	39.9	NT	NT	NT	NT	NT	32.0
Imipenem	40.9	45.2	35.2	33.1	43.1	84.4	70.1	50.3
Amoxycillin+Clavulanicacid	NT	NT	NT	0.4	1.9	2	16.1	6.7
Piperacillin+Tazobactum	NT	NT	NT	75.4	62.8	84.4	70.7	73.3
Ticarcillin+Clavulanic acid	NT	NT	NT	30.1	27.1	18.9	26.8	25.7
Meropenem	NT	NT	NT	55.1	57.8	36.2	36.9	46.5
Aztreonam	NT	NT	NT	NT	NT	65.2	74.7	70.0

Abbreviations: NT-Not Tested; NA Not Available.

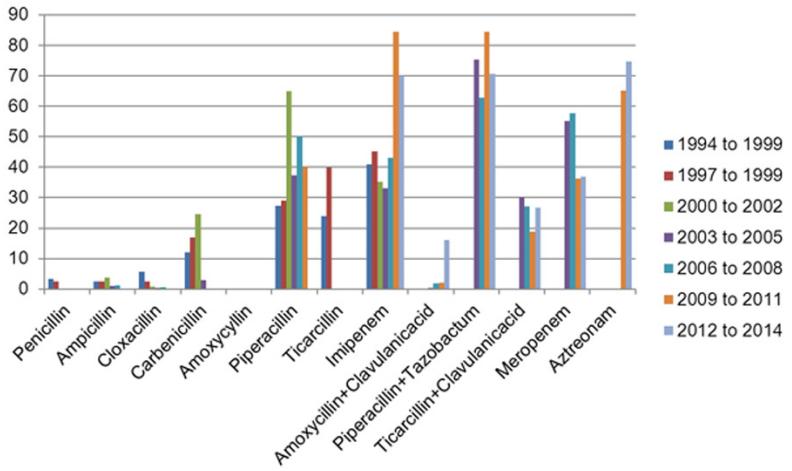

Figure 1. Graphical representation of antibiotic sensitivity (%) of Penicillin and Carbapenems.

organisms including Enterobacter species has been established and recognized [3].

Burn injury colonization, serious wound infection and sepsis, by nosocomial pathogen, Pseudomona aeruginosa is recognized as major cause of morbidity and mortality [4].

Materials and methods

Between 1994 and 2014, 2399 pediatric patients in the age group between 1 month and 15 years were admitted to the Burns unit. Microbiological samples were collected (11402 swabs were processed) and their bacteriology and antibiotic sensitivities recorded.

Wound treatment

Closed dressings using silver sulphadiazine ointment were used in all patients without exception. The burn wounds were washed daily to remove necrotic tissue and the remnants of the previous day's ointment.

Procedure for wound sampling

Microbial colonization of all wounds was studied from the time of admission to discharge. On admission, the sampling procedure included swabs that were taken from clinically deep areas of the burn wound prior to any cleansing. Swabs were taken twice weekly. The bandages were removed and the wounds are washed. The wounds were swabbed and cultured as follows: A sterile cotton swab is moistened with sterile normal saline. This swab is rubbed onto the burn wound surface. Swabs are taken from areas which appear deep, areas with discharge or thick eschar. The swabs are then sent immediately for culture.

Table 2. Antibiotic sensitivity (%) of Cephalosporin

YEAR	1994-1996	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	Average
Total Isolates	709	1231	1197	935	603	677	210	
Antibiotics	%	%	%	%	%	%	%	%
Cephalexin	12.7	6.9	2	0.6	1	NT	NT	4.6
Cephazolin	29.5	13.2	3.5	4.1	2.1	1.5	NT	9.0
Cefuroxime	45.5	39.1	18.4	2.3	3.8	0.9	7	16.7
Cephadroxyl	35.8	10	1.9	2.9	3.1	NT	NT	10.7
Cefotaxime	43	40.8	27.1	8.7	12.6	18.3	60.4	30.1
Ceftazidime	48.2	41.5	59.9	13.9	7.1	13.9	NT	30.8
Ceftriaxone	49.3	43.8	25.7	8.4	5	3.7	NT	22.7
Ceftizoxime	58.7	41.1	19.9	45.8	32.2	16.5	21.6	33.7
Cefoperazone	66.2	46.9	67.5	41.4	40	15.9	NT	46.3
Cefixime	NT	NT	13.4	1.7	3.1	0.5	NT	4.7
Cefpirome	NT	NT	66.7	17.5	6.2	7	NT	24.4
Cefepime	NT	NT	NT	39.8	61.1	28.5	NT	43.1
Cefoperazone+sulbactum	NT	NT	74.1	69.8	82.7	75.4	66.2	73.6
Cefotaxime+sulbactum	NT	NT	NT	36.8	32.2	28.3	NT	32.4
Ceftriaxone+sulbactum	NT	NT	NT	30.2	38.2	16.5	37.7	30.7

Abbreviations: NT-Not Tested; NA Not Available.

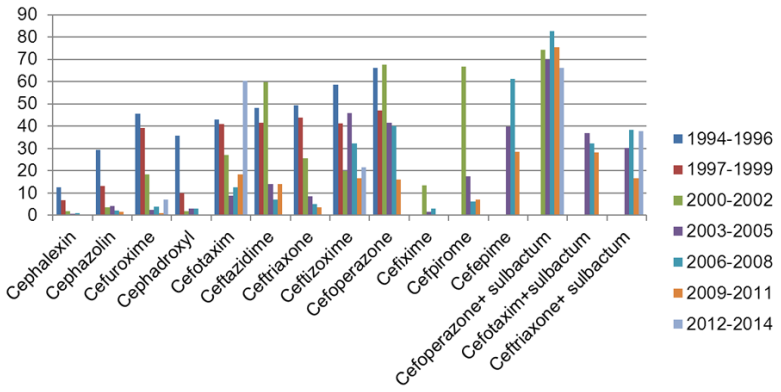

Figure 2. Graphical representation of antibiotic sensitivity (%) of Cephalosporins.

Microbiology

The swabs are transported to the laboratory for processing immediately. They are streaked onto a differential medium (e.g.; MacConkey agar) and an enriched medium (e.g. blood agar). Isolation is carried out by the conventional T-method using sterile nichrome loop. These plates are incubated at 37°C for 16-18 h. The basic aim was to isolate the organisms predominant on the burn wound and determine their sensitivity to various antibiotics for clinical purposes.

Antibiotic sensitivity of isolates obtained from the burn wound was carried out by Agar disc diffusion method (Kirby Bauer method) [5]. Sterile commercially available filter paper discs, onto which a definite amount of antibiotic has been absorbed, are used. Since the antibiotic in the disc tends to diffuse more onto the surface of the agar than into the deeper layers, the plate is surface spread with the organisms. A broth culture of the isolate is prepared using sterile peptone water comparable to 0.5 McFarland's turbidity standard (i.e. 1×10^7 to 1×10^8 organisms/ml). Approximately 0.2 ml of this broth culture is surface spread onto sterile Mueller Hilton agar so as to get a matt growth. Sterile antibiotic discs are equidistantly placed on these plates and gently pressed onto the medium with the help of sterile forceps to ensure complete contact with the agar surface. The plates are incubated at 37°C for 16 to 18 h. Zone of inhibition was measured in millimeters and sensitivity reported.

Table 3. Antibiotic sensitivity (%) of Aminoglycoside

Year	1994-1996	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	Average
Total Isolates	709	1231	1197	935	603	677	210	
Antibiotics	%	%	%	%	%	%	%	%
Streptomycin	29.5	14.9	NT	NT	NT	NT	NT	22.2
Gentamycin	24.7	15.8	9.7	3.2	11.9	16.7	33.5	16.5
Tobramycin	17.5	16.6	26	7.3	8.8	17.4	37.2	18.7
Amikacin	66.8	48.7	73.8	42	53.6	68.6	60.1	59.1
Netilmycin	35.4	41.6	65.9	39.3	44.8	49.2	59.2	48.0

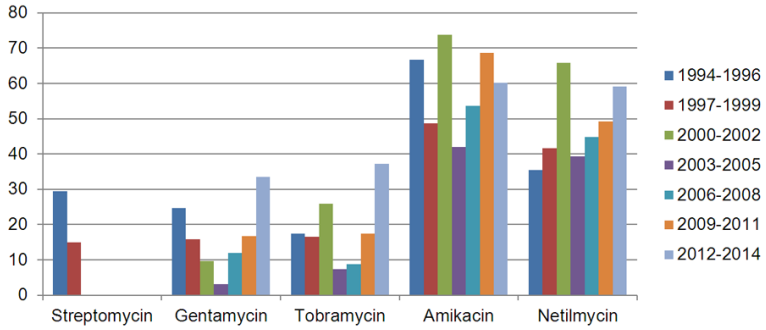

Figure 3. Graphical representation of antibiotic sensitivity (%) of Aminoglycosides.

Observations

Among 11493 microbiological samples which were taken during the study period, 17507 bacterial strains were isolated and the frequency of P.aeroginosa (5562) was found to be 31.8%.

Broadly, it was observed that Pseudomonas showed a good average sensitivity (%) to the following antibiotics; Piperacillin+Tazobactam (73.3%), Meropenem (46.5%), Imipenem (50.3%), Astreonam (70%), Amikacin (59.1%), Netlimycin (48%), Azithromycin (62%), Cefoperazone+Sulbactam (73.6%), Colistin (47.3%).

Results

*Penicillins (**Table 1, Figure 1**)*

Carbenicillin gave 24.6% sensitivity in 2002 but following that, the sensitivity started dropping alarmingly. We would like to draw your attention to the fact that while we rarely subscribe to the use of Carbenicillin, the rapid fall in sensitivity alerts us to the fact that it is rampantly and probably improperly used outside our hospital and thus contributes to the microorganisms gaining resistance. It is thus a reflection of the usage in society at large. In our series, the addition of beta lactamase inhibitors increased the sensitivities in the case of Piperacillin and Tazobactam but decreased sensitivity was seen when Ticarcillin was combined with Clavulanic acid. As reported by Simon et al, resistance to Piperacillin develops during therapy and has led to an admonition that Piperacillin should not be used as a single agent [6].

*Carbapenems (**Table 1, Figure 1**)*

Meropenem and Imipenem continue to show good sensitivities in our series. In our unit, these are antibiotics are used only when resistance has been documented to all other antibiotics.

Astreonam, a new class of beta lactam antibiotics, the monobactams was introduced in our unit in 2009 and showed a good sensitivity to Pseudomonas.

*Cephalosporins (**Table 2, Figure 2**)*

Tredget et al [7] state that only Ceftazidime and Cefaperazone have been found to have anti-Pseudomonal activity. In our series we have an excellent example of how good antibiotic have lost their sensitivity over a period of time. To begin with Cefoperazone and Ceftazidime did show good sensitivity, however we lost sensitivity of these antibiotics in a span of a few years. Later addition of Sulbactum to Cefaperazone did boost the sensitivity rating.

*Aminoglycosides (**Table 3, Figure 3**)*

Though Tredget et al [7] report that 91% of Pseudomonas isolates from his unit are sensi-

Table 4. Antibiotic sensitivity (%) of Fluroquinolones

Year	1994-1996	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	Average
Total Isolates	709	1231	1197	935	603	677	210	
Antibiotics	%	%	%	%	%	%	%	%
Ofloxacin	66.4	36.2	30.7	10.3	8.5	3.8	NT	26.0
Pefloxacin	45.3	26.2	19.9	6.4	3.8	2.4	NT	17.3
Norfloxacin	48.8	33.6	25.5	6.2	8.5	16	NT	23.1
Ciprofloxacin	57.5	44.2	42.4	13.8	24.7	60.7	66.9	44.3
Sparfloxacin	NT	NT	42.9	17.5	21.8	21.4	30.6	26.8
Lomefloxacin	NT	NT	31.3	12.4	19	15.2	26.6	20.9
Gatifloxacin	NT	NT	NT	55.1	45.3	58.2	39.8	49.6

Abbreviations: NT-Not Tested; NA Not Available.

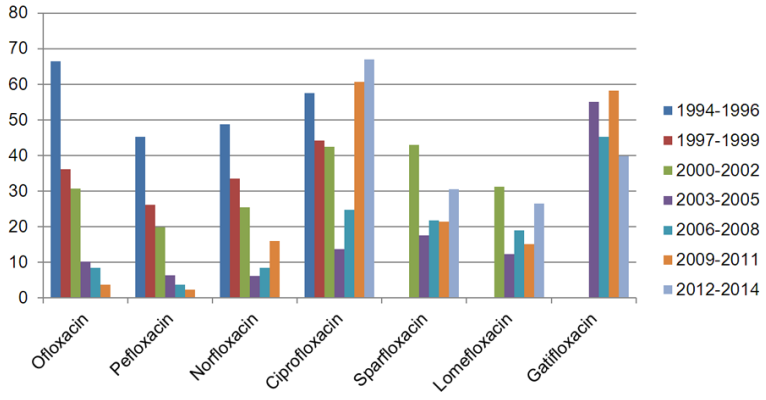

Figure 4. Graphical representation of antibiotic sensitivity (%) of Fluroquinolones.

tive to Tobramycin and Ciofu mentioning that resistance to Tobramycin developed less than other non-aminoglycoside antibiotics [8], we found that our isolates showed good sensitivity to Amikacin against Tobramycin. Tredget [7] also rightly suggests that as resistance is related in part to permeability, resistance to Tobramycin or Gentamycin does not mean resistance to Amikacin and separate sensitivity testing should be performed. In our series, Amikacin and Netilmycin top the charts with good sensitivities. Nasilowski et al showed that their strains of Pseudomonas were sensitive to Gentamycin and Colistin in 100% and to Streptomycin in 57% [9]. In Tahlan's series, gentamycin was the most effective drug against Pseudomonas (85%) [10]. Malcolm Eve and Settle [11] make a point with special reference to pediatric patients - though serum levels of Amikacin were found to be adequate with standard doses in the majority of adults, greater doses were required than those recommended by the manufacturer in children. They also recommend Amikacin as the first choice aminoglycoside where the sensitivity pattern is unknown but Gentamycin resistance is known to occur, or where the organism is known to be resistant to gentamycin, and should also be considered in life threatening infections even with gentamycin-sensitive organisms.

*Fluoroquinolones (**Table 4, Figure 4**)*

Initially, the quinolones were sparingly used in pediatric patients by virtue of their ability to retard growth. Ciprofloxacin retained good sensitivity owing to its minimalistic use in pediatric patients. Its sensitivity started dropping between 2003-2005. However it gained its sensitivity thereafter. A similar observation has been made by Settle [12]. Gatifloxacin introduced in our unit in 2003 showed good sensitivity to begin with, however sensitivity decreased over time.

*Macrolides (**Table 5, Figure 5**)*

Azithromycin leads the pack showing a sensitivity to 74.7% isolates in 2014.

*Other antibiotics (**Table 6, Figure 6**)*

Colistin showed good sensitivity in 2014. This antibiotic was discontinued in our unit in 2000 due to poor sensitivity. However when it was tested in 2009, it had regained its sensitivity thus proving that lack of use of an antibiotic

Table 5. Antibiotic sensitivity (%) of Macrolides

Year	1994-1996	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	Average
Total Isolates	709	1231	1197	935	603	677	210	
Antibiotics	%	%	%	%	%	%	%	%
Erythromycin	23.4	27.3	7	0.6	2.1	11.1	9.2	11.5
Azithromycin	NT	NT	66.4	37.5	66.4	65.2	74.7	62.0
Roxithromycin	NT	NT	3.5	1.9	1.7	11.8	4.6	4.7
Clarithromycin	NT	NT	14.1	9.2	4	2.8	9.1	7.8

Abbreviations: NT-Not Tested; NA Not Available.

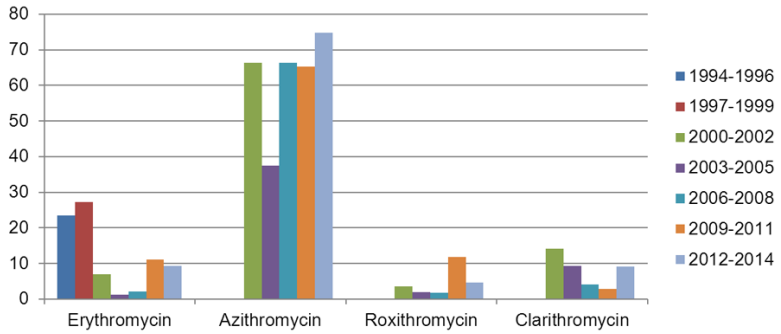

Figure 5. Graphical representation of antibiotic sensitivity (%) of Macrolids.

can have a considerable effect on the sensitivity pattern of antibiotics.

Discussion

Colebrook and associates showed that only a few patients were colonized with P.aeroginosa if admitted to hospital within a few hours of their injury. There was an increase in the acquisition of the organism when patient turnover was increased, some overcrowding being common at busy times [13]. Sutter and Hurst [14] have shown that the majority of Pseudomonas infections in burns are due to cross infection in hospital. Phage typing has also shown that most infections are caused by Pseudomonas strains previously detected in other patients in the same hospital wards. Yemul and Sengupta maintain that Pseudomonas infections occurred from several different sources, namely, endogenous infection from feces and cross infections from nursing staff and the hospital environment. In their series, no one type of cross infection could be pinpointed as important and all sources were equally important [15].

Laboratory studies can assist in determining antibiotic sensitivities for most bacteria. Unstable patients often require empiric therapy guided by initial cultures taken on admission. Initial antibiotic therapy is based on these swabs and tailored once further sensitivities become available. It is important to maintain continuous records of the bacteria isolated along with their sensitivity pattern to assist in antibiotic selection. These should be developed for individual hospitals.

Unfortunately, with the development of multi drug resistant pseudomonas, the choice of antibiotics becomes more difficult. Pruitt showed that the burn wound is relatively free of bacteria during the first 24 hours after injury. They get colonized over the ensuing days or weeks by Gram negative bacteria. By the third week post-burn, the wounds of over 70% of patients were colonized by Pseudomonas if topical therapy was not employed [1].

Conclusion

The development of a policy for the use of antibiotics in a burn unit is important. They must be used for a precise purpose, be it for prophylaxis or therapy of established infection. The duration of antibiotic administration must be sufficient for the required purpose without it being excessively long. Sub inhibitory concentrations of antibiotics over a short period allow resistance to develop in the target microbes, whereas long durations of therapy will allow microbes resistant to the antibiotics to establish themselves on the burns wound. It is also useful and important to remember that pharmacokinetics of many antibiotics are altered in the burns patient and that there is considerable and significant inter subject variation. It is therefore

Table 6. Percentage antibiotic sensitivity of other antibiotics

Year	1994-1996	1997-1999	2000-2002	2003-2005	2006-2008	2009-2011	2012-2014	Average
Total Isolates	709	1231	1197	935	603	677	210	
Antiobiotics	%	%	%	%	%	%	%	%
Colistin	34.9	15.4	NT	NT	NT	55.4	83.3	47.3
Co-trimoxazole	15.7	10.9	5	19.9	3.1	0.2	9.1	9.1
Tetracycline	22	14.6	11	6.6	3.3	5.4	19.1	11.7
Chloramphenicol	29.5	23.3	21.8	15.3	9.7	5.7	25	18.6
Metronidazole	1.8	0	0	0.2	0.9	0	0	0.4
Clindamycin	NT	NT	6.2	1.9	2.1	0.6	0.7	2.3
Spiramycin	NT	NT	2.6	1.1	NT	NT	NT	1.9
Tigecycline	NT	NT	NT	NT	NT	NT	38.9	38.9

Abbreviations: NT-Not Tested; NA Not Available.

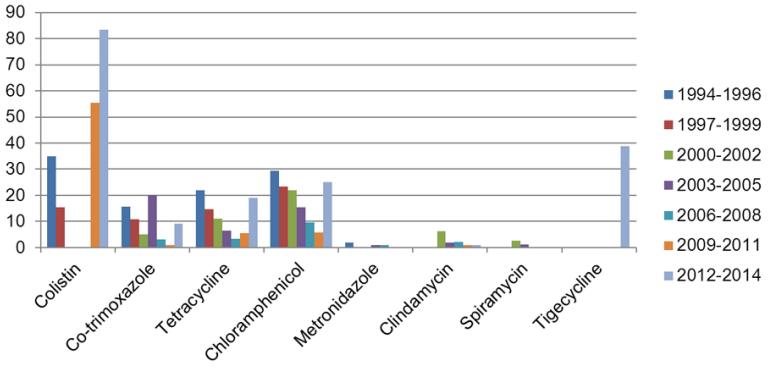

Figure 6. Graphical representation of antibiotic sensitivity (%) of other antibiotics.

vital that antimicrobial therapy of each burn patient is individually assessed.

In our unit, antibiotics are administered only when there is suspicion of sepsis in the burn patient. In this situation, antibiotics are selected based on ongoing surveillance of the patient's burnt areas. If there is no response or if results of clinical samples suggest, antimicrobials are changed as deemed fit and patient responses are judged. A similar protocol is followed at the Burns unit in Kuwait [16].

Pseudomonas is very resistant to most antibiotics and this resistance develops very rapidly. The use of antibiotics to control acquisition of infection is not reasonable or cost effective [17].

Bruce G. MacMillan states that the most common sources of infection found during his 10 year study have been the patient, the personnel, sinks, floors, diet, soaps, respirators, warming and cooling equipment and hydrotherapy equipment. He reinforces the belief that elaborate equipment is not necessary to control infection within the burns unit and reinforces the importance of strict adherence to proven principles of isolation for the prevention of cross infection in intensive care units charged with the care of critically ill patients [18].

Our best hope lies in being vigilant to the earliest signs of sepsis, bacteriological surveillance of wound swabs and appropriate antimicrobial treatment as and when indicated.

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Disclosure of conflict of interest

None.

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