

# 12 Years Analysis of Bacterial Isolates and Their Antibiotics Sensitivity in a Tertiary Care Burns Unit

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## Abstract

The aim of this study was to review the changes in distribution of bacterial populations and their antibiotic sensitivity over 12 years in a tertiary care burn unit. Understanding the periodic variation of isolated microorganisms and their antibiotic sensitivity helps in selecting the appropriate antimicrobial therapy before culture and sensitivity are reported. It also aids the design of antibiotics protocols. The study was retrospective. The data were obtained from the computerised hospital medical record system and the burn unit records. Overall, *Pseudomonas aeruginosa* was the most commonly isolated microorganism followed by *Staphylococcus aureus*, *Meticillin-resistant Staphylococcus aureus* (MRSA), and the genus *Acinetobacter*. *Acinetobacter* isolation rose rapidly and became more prevalent than *P. aeruginosa* over the last three years. Other organisms became isolated more frequently, such as *Klebsiella pneumoniae*, but their overall prevalence was low. *Pseudomonas* species frequency of isolation declined. *P. aeruginosa*, MRSA, and other microorganisms showed increasing sensitivity to a number of antibiotics. MRSA remained highly sensitive to vancomycin, and *Acinetobacter* showed high resistance to all antibiotics tested except colistin. *K. pneumoniae* was highly resistant to most of the antibiotics tested except the carbapenems, but the resistance to carbapenems increased over time.

## Keywords

Burn, Microorganism, Antibiotic, Resistance, Sensitivity

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## 1. Introduction

Infection is a frequent complication of burn injury. It is responsible for 75% of deaths in patients who sustain burns [1]. The increased risk of infection associated with burns is due to a number of factors. Two important factors are severe immunosuppression and loss of the skin barrier [1] [2]. When the skin barrier is lost, a burn wound is created. The burn wound is a good environment for microorganism growth because it is moist and contains necrotic tissues [3]-[5]. As a result, it becomes colonized rapidly by the skin flora, the bacteria that are usually present in the sweat glands and hair follicles.

The respiratory and gastrointestinal tracts can become additional sources of microorganisms. Invasive procedures, such as endotracheal intubation, invasive central venous or arterial lines, and urinary catheterization also increase the chances of infection [6]. Furthermore, organisms present in the hospital environment may be spread to the patient by health workers [3] [7] [8].

The pattern of bacterial isolates and antibiotic sensitivity is not constant, but changes with time. Understanding the periodic variation of isolated microorganisms and their antibiotic sensitivity helps in selecting the appropriate antimicrobial therapy before culture and sensitivity are reported [2] [9]. It also aids the design of antibiotics protocols.

The changing population patterns of microorganisms and their antibiotic sensitivities have become more complicated in recent years by the rise of antibiotic resistant microorganisms. The genus *Acinetobacter* has become a major problem in many burn units globally. Unfortunately, there are no new antibiotics to combat these resistant microorganisms. As a result, older, abandoned antibiotics have again become important in the management of antibiotic resistant microbes [10].

This report describes changes in the distribution of bacterial populations and their antibiotic sensitivity over 12 years at Khoula hospital burns unit. Khoula hospital is a tertiary care hospital and the burns unit is the only tertiary care burns unit in Sultanate of Oman.

## 2. Methods

The study was retrospective over 12 years and included all patients who were admitted to the Khoula Hospital burns unit from 01.01.2003 to 31.12.2014 and had positive cultures. The laboratory data were obtained from the computerised hospital medical record system [Al-Shifa Healthcare Information System] and the demographic data were obtained from burns unit records. Swab cultures, biopsy cultures, blood cultures, line cultures, secretion cultures and urine cultures were included. Patient demographic data and bacteriology reports of various cultures and their antibiotic sensitivities were reviewed. The data were entered into a spreadsheet and analysed using the Microsoft Office Excel 2007 program. We scored the presence of bacteria cultured from each patient during their visit. For example, if the patient had three consecutive positive swabs for a particular type of bacteria it was counted as one. The twelve-year period was divided into four three-year periods: 2003-2005, 2006-2008, 2009-2011 and 2012-2014. The number of admissions per year during the period of study was similar (mean/year: 191.25). The study was conducted with the approval of the hospital ethics committee.

## 3. Results

### 3.1. Demographic Data (Table 1)

A total of 2295 patients were admitted. There were 1402 (61%) males and 893 (39%) females. The number of adults was 1205 (53%) and the number of children was 1090 (47%). Scald was the most common cause of burns (1102 patients), followed by flame (860 patients), 316 (14%) patients required ventilation, 138 (6%) patients died.

### 3.2. Bacterial Prevalence

The total number of patients who had positive cultures was 1206 (53%). The total number of isolates was 7110. Fifty-seven isolates were excluded from the analysis as they were isolated sporadically and we considered them not significant. Therefore, 7053 isolates were included in the analysis of bacterial prevalence. Overall, *Pseudomonas aeruginosa* was the most common isolate (1607) followed by *Staphylococcus aureus* (1093), Meticillin resistant *Staphylococcus aureus* (MRSA) (1013), *Acinetobacter* (882), other *Klebsiella* species (537), *Staphylococcus epidermidis* (409), other *Pseudomonas* species (363), *Escherichia coli* (303), *Enterococci* (282),

**Table 1.** Demographic data.

	2003-2005	%	2006-2008	%	2009-2011	%	2012-2014	%	Total	%
Admission	530		541		602		622		2295	
Inhalation	66	12.5	69	12.8	82	13.6	99	15.9	316	13.8
Death	31	5.8	22	4.1	40	6.6	45	7.2	138	6.0
Male	335	63.2	319	59.0	362	60.1	386	62.1	1402	61.1
Female	195	36.8	222	41.0	240	39.9	236	37.9	893	38.9
Adults	251	47.4	282	52.1	329	54.7	343	55.1	1205	52.5
Children	279	52.6	259	47.9	273	45.3	279	44.9	1090	47.5
TBSA Burned										
≤10%	308	58.1	342	63.2	359	59.6	359	57.7	1368	59.6
11% - 20%	117	22.1	105	19.4	105	17.4	137	22.0	464	20.2
21% - 50%	77	14.5	65	12.0	108	17.9	93	15.0	343	14.9
>51%	28	5.3	29	5.4	30	5.0	33	5.3	120	5.2
Cause of Burn										
Scald	281	53.0	269	49.7	271	45.0	281	45.2	1102	48.0
Flame	186	35.1	182	33.6	248	41.2	244	39.2	860	37.5
Electrical	33	6.2	38	7.0	35	5.8	43	6.9	149	6.5
Chemical	15	2.8	25	4.6	25	4.2	26	4.2	91	4.0
Friction	1	0.2	4	0.7	5	0.8	5	0.8	15	0.7
Sun	1	0.2	1	0.2	0	0.0	0	0.0	2	0.1
Contact	11	2.1	22	4.1	17	2.8	23	3.7	73	3.2
Drug	2	0.4	0	0.0	1	0.2	0	0.0	3	0.1
Total	530		541		602		622		2295	

*Klebsiella pneumoniae* (167) and *Streptococcus* (143). Other organisms that were less commonly isolated were *Enterobacter* (88), coliform bacteria (70), *Proteus* (69) and *Serratia* (27).

### 3.3. Pattern of Isolates (Figure 1)

#### 3.3.1. Gram-Positive

Meticillin resistant *Staphylococcus aureus* (MRSA) was the most prevalent organism during the first period (2003-2005), where it was isolated 660 times. Subsequently, there was a sudden drop to 106 isolates in the second period and it remained static thereafter. During 2003-2005, MRSA was more prevalent than *P. aeruginosa*. *S. aureus* isolation was static throughout. The *Staphylococcus epidermidis* isolation rate rose progressively, from 71 times during 2003-2005 to 176 times during 2012-2014.

#### 3.3.2 Gram-Negative

*Acinetobacter* was isolated infrequently during the period of 2003-2005 (31 isolates); however, after 2006 there was a dramatic rise, reaching 483 isolates by the period of 2012-2014. *P. aeruginosa* was the most commonly isolated Gram-negative organism until the period of 2012-2014, when *Acinetobacter* became the most prevalent,

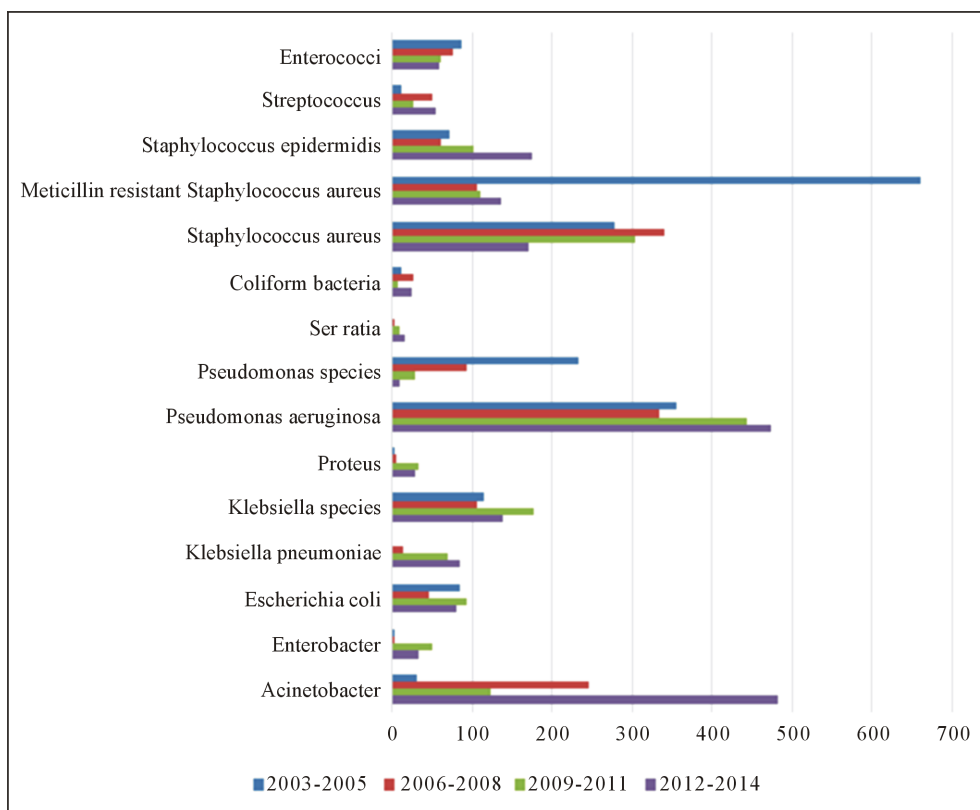


Figure 1. Pattern of bacterial isolates.

but the frequency of *P. aeruginosa* isolation continued to rise overall. The isolation of other *Pseudomonas* species reduced gradually over the four periods, dropping to 10 isolates only during the 2012-2014 period. *K. pneumoniae* was never isolated during the period of 2003-2005 and rose gradually to 84 isolates during the period of 2012-2014. Other *Klebsiella* species did not show any notable variation over the twelve-year period. *Escherichia coli* isolation was static over the study period.

### 3.4. Pattern of Antibiotic Sensitivity

#### 3.4.1 Gram-Positive (Table 2)

*S. aureus* was always sensitive to all groups of antibiotics. Methicillin resistant *Staphylococcus aureus* (MRSA) was highly resistant to erythromycin, fucidate and gentamicin during the period of 2003-2005. Subsequently there was a gradual increase in sensitivity. Throughout the four periods, MRSA was highly sensitive to glycopeptides. Linezolid, rifampicin, and tigecycline were tested during the last period only and the sensitivity of MRSA to these antibiotics was 100.0%.

*S. epidermidis* showed decreasing sensitivity to penicillins, lowering to about 10.0% sensitivity in 2012-2014. It was 100.0% sensitive to vancomycin during the 12 years while sensitivity to teicoplanin ranged from 95.0% to 100.0% during the period of study. Linezolid and rifampicin testing carried out in last three years of the study demonstrated 100.0% sensitivity of *S. epidermidis* to these antibiotics.

*Streptococcus* was 100.0% sensitive to cephalosporins until the final study period, when the sensitivity dropped to about 50.0%.

#### 3.4.2. Gram-Negative (Table 3)

*P. aeruginosa* was highly sensitive to the piperacillin/tazobactam combination antibiotic throughout the study period. It was highly resistant to the cephalosporin group during the first two periods (2003-2005 and 2006-2008) whereas the sensitivity increased tremendously during the last two periods (2009-2011 and 2012-2014). With regard to the aminoglycoside and carbapenem groups, the period of 2006-2008 showed high resistance, but

subsequently there was a high sensitivity. Quinolone sensitivity also increased during the last two periods. Colistin was tested during the last two periods and the sensitivity was 100.0%.

*Acinetobacter* was 38.7% sensitive to the piperacillin/tazobactam combination antibiotic during the period of 2003-2005. This dropped to 6.1% in 2012-2014. It did not show any significant sensitivity to cephalosporins or aminoglycosides. Sensitivity to ciprofloxacin dropped from 45.2% to 6.5%. It was highly sensitive to imipenem (96.2%) during the first period, but there was rise in resistance from 2006 onwards. With regard to colistin, the sensitivity was 100.0% during the period 2009-2011, and 99.2% during 2012-2014.

*Klebsiella* species demonstrated high sensitivity to piperacillin/tazobactam throughout the study period whereas it showed low sensitivity to amoxicillin-clavulanate and ampicillin. *Klebsiella* sensitivity to cephalosporins rose over the 12-year period. A striking finding was that sensitivity to ceftazidime was 3.4% during 2003-2006 and 97.0% during 2012-2014. *Klebsiella* species were also becoming more sensitive to aminoglycosides. The sensitivity to carbapenems and glycopeptides was stable and almost 100.0% throughout the 12-year period.

*E. coli* was highly sensitive to carbapenems at all times tested and in the period of 2012-2014, the sensitivity was 100.0%. Also, *E. coli* was highly sensitive to piperacillin/tazobactam and the aminoglycoside group at all times tested. Overall, *E. coli* sensitivity to cephalosporins reduced over time.

**Table 2.** Antibiotics sensitivity of Gram +ve bacteria.

	Enterococci				MRSA				<i>Staphylococcus aureus</i>				<i>Staphylococcus epidermidis</i>				<i>Streptococcus</i>			
	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %
Amoxicillin + Clavulanate	100	94.4	100	100	-	-	-	-	-	100	-	-	-	-	-	-	100	93.9	92.3	100
Ampicillin	74.1	80.3	86.9	85.7	50	-	-	-	-	-	-	-	-	-	-	-	90.9	77.6	85.2	80
Meticillin	-	-	-	-	-	1.9	-	-	98.9	99.1	100	99.4	43.7	28.3	21	10.8	-	-	-	-
Penicillin	1.2	1.3	25.4	75.4	-	-	-	-	5	5.6	7	4.8	5.6	1.7	6	2.3	55.6	19.1	55.6	64.8
Cloxacillin	-	-	-	-	-	2.2	-	-	99.6	99.1	99.7	98.8	46.7	32.7	18.8	10.3	-	-	-	-
Piperacillin + Tazobactam	-	100	100	100	-	-	-	-	-	-	-	-	-	-	-	-	-	100	-	-
Ceftriaxone	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	100	100	100	50
Cefuroxime	-	-	-	100	-	-	-	-	-	-	-	-	-	-	-	-	100	-	100	66.7
ciprofloxacin	100	-	100	100	-	-	-	68	100	-	-	100	100	-	-	-	-	-	-	-
Erythromycin	100	33.3	37.5	54	1.6	69.1	64	80.6	96	96.1	95.7	95.7	35.7	16.4	22.8	18.7	100	50	73.1	35.4
fusidate	-	-	-	-	2	51	36.9	46	96	85.5	52.5	44.2	42.3	41.7	26	18.2	-	-	-	-
Clindamycin	-	-	-	-	-	-	-	92.6	-	-	-	100	-	-	-	80.3	-	-	-	-
Amikacin	100	-	100	100	50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gentamicin	100	-	-	100	1.8	80.8	74.3	-	96.4	96.6	97	100	31	25.9	41.5	-	-	-	-	-
Trimrthoprim + sulfamet	33.3	-	40	100	97.9	84	83.5	92.1	89.1	95	92	99.4	49.3	63.9	53.1	50.3	50	8.9	31.8	50
Teicoplanin	100	100	100	89.3	99	-	98.1	100	88.9	-	97.8	100	94.3	-	100	90	-	100	100	100
Vancomycin	100	100	100	90	100	99	100	99.2	100	98.6	100	100	100	100	100	100	-	100	100	100
Linezolid	-	-	-	100	-	-	100	100	-	-	100	100	-	-	-	100	-	-	-	100

**Table 3.** Antibiotics sensitivity of Gram –ve bacteria.

	<i>Acinetobacter</i>				Coliform bacteria				Enterobacter				<i>Escherichia coli</i>				<i>Klebsiella pneumoniae</i>			
	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %
Amoxicillin + Clavulanate	-	-	-	-	36.4	24	14.3	20	100	-	2	6.1	48.8	53.3	43.3	49.4	-	-	17.4	17.1
Ampicillin	-	-	-	-	-	3.8	-	4.2	50	-	-	3	7.4	20	5.2	17.5	-	-	-	-
Piperacillin + Tazobactam	38.7	4.5	26.9	6.1	100	85.7	83.3	79.2	100	100	89.4	96.8	73.1	86.8	92.7	97.3	-	64.3	87.9	69.6
Cefepime	12.9	0.8	26.9	5.6	100	73.3	57.1	95.5	50	100	97.6	90.9	87.2	53.3	66.3	65	-	10	14.8	12.1
Cefotaxime	-	-	-	-	-	40	57.1	80	-	100	60	84.8	100	31.6	55.9	62.5	-	-	7.5	11
Ceftazidime	-	18.2	30.1	6.1	-	31.3	57.1	87.5	100	100	62	86.7	66.7	35.3	66.7	63.4	-	-	7.2	11.5
Ceftriaxone	-	-	-	-	83.3	40	57.1	80	100	100	60	87.9	81.6	35.3	54.2	61.3	-	-	7.2	11
Cefuroxime	-	-	-	-	75	40.9	28.6	68	100	100	42	75.8	87.7	41.9	55.7	57.5	-	-	7.2	11
Cephadrine	-	-	-	-	18.2	30.4	14.3	26.7	25	-	-	-	81.5	42.9	40.2	39.1	-	-	5.8	15.4
Ciprofloxacin	45.2	2.9	32.8	6.5	100	66.7	71.4	84	100	100	85.7	87.9	61.3	65	70.1	63.8	-	14.3	60.9	48.8
Amikacin	22.6	0.8	28.7	8.6	100	89.5	100	95.8	100	100	90	96.9	84.5	90.5	97.9	97.3	-	78.6	92.8	69.3
Gentamicin	-	1.3	23.8	7.3	83.3	47.8	57.1	84	100	100	64	90.9	74.4	74.3	45.2	72.2	-	-	30.4	34.6
Imipenem	96.2	10.5	28.7	6.3	100	90.9	100	96	-	100	100	100	90	100	100	100	-	100	100	74.7
Meropenem	37.5	4.6	25.4	6.7	100	90	100	100	-	100	100	100	91.3	78.6	100	100	-	100	100	76.7
Trimethoprim + Sulfamethoxazole	-	-	50	6.4	91.7	24	33.3	70.8	100	100	58.3	78.1	-	-	-	-	-	-	25.8	21.1
Colistin	-	-	100	99.2	-	-	-	-	-	-	-	100	29.6	50	30.5	35.9	-	-	-	-

	<i>Klebsiella species</i>				Proteus				<i>Pseudomonas aeruginosa</i>				<i>Pseudomonas species</i>				<i>Serratia</i>			
	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %	1st %	2nd %	3th %	4th %
Amoxicillin + Clavulanate	48.7	26.7	35.8	63.5	100	100	85.7	85.7	-	-	-	-	-	-	-	-	-	-	-	-
Ampicillin	3.5	1	0.6	1.5	100	25	22.9	17.9	-	-	-	-	-	-	-	-	-	-	-	-
Piperacillin + Tazobactam	82.8	71.9	88.3	93.2	-	100	100	93.1	85.4	83.8	93.9	81.8	82	87.1	81.5	75	-	100	88.9	87.5
Cefepime	65.6	63.3	89.4	98.3	-	100	94.4	49.3	42.7	94	84.4	42.3	33.3	73.9	77.8	-	100	88.9	92.9	
Cefotaxime	33.3	24.6	74.4	94.2	-	-	100	96.4	-	-	-	-	-	-	-	-	-	-	77.8	93.8
Ceftazidime	3.4	25	75.7	97	-	-	100	96.3	26.6	25.4	90.8	81.4	23	20.6	82.1	88.9	-	50	88.9	93.8
Ceftriaxone	39.1	23	74	94.2	-	-	100	96.4	-	-	-	-	-	-	-	-	-	-	77.8	93.8
Cefuroxime	75	31.2	69.3	89.1	100	-	91.4	88.9	-	-	-	-	100	-	-	-	-	-	-	-
Cephadrine	51.3	25.8	36.7	69.3	-	20	20	41.2	-	-	-	-	-	-	-	-	-	-	-	18.2
Ciprofloxacin	79.6	76.3	84.1	96.3	-	40	100	88.5	67.9	63.9	93	91.4	54.3	42.2	82.1	88.9	-	100	88.9	100

## Continued

Amikacin	93.8	78.2	94.3	99.2	-	100	100	100	70.8	47.7	86.1	90.5	65.2	43.2	71.4	88.9	-	50	100	100
Gentamicin	68.8	51.6	68.8	94.8	100	33.3	97.1	85.7	59	41.8	75.5	87.5	43.5	34.1	50	77.8	-	100	77.8	100
Imipenem	100	95.2	99.4	100	-	-	100	100	55.2	34.8	86.3	76.2	64	40	80.8	70	-	100	100	100
Meropenem	100	95.6	100	100	-	-	100	100	52.8	31.9	92.5	80.3	47.9	32.1	82.1	77.8	-	100	100	100
Trimethoprim + Sulfamethoxazole	47.6	33	64	78.8	100	40	74.2	72	-	-	20	-	-	-	-	-	-	100	77.8	100
Colistin	-	-	-	-	-	-	-	-	-	-	100	100	-	-	-	-	-	-	-	-

*Pseudomonas* species demonstrated intermediate sensitivity to all tested antibiotics, *Enterococci* did not show any significant resistance and *Klebsiella pneumoniae* was highly resistant to most of the antibiotics except the carbapenems. During the period of 2012-2014, the sensitivity to carbapenems declined. *K. pneumoniae* demonstrated intermediate sensitivity to piperacillin/tazobactam and amikacin.

#### 4. Discussion

*P. aeruginosa*, *S. aureus* and MRSA are the most commonly isolated microorganisms worldwide [9] [11]-[18]. This pattern of distribution appears to be constant and does not change over time. In a review of 50 years of isolates, *S. aureus* and *P. aeruginosa* were the most commonly isolated organisms [5]. This is a global phenomenon and the pattern in our burns unit was not different.

On the other hand, there has been a sharp rise in *Acinetobacter* isolation over recent years. *Acinetobacter* was rarely isolated before 1970 [5]. In older reports, *Acinetobacter* either did not grow at all [19], or was isolated but its presence was not that significant. *Acinetobacter* is now recognized as a microorganism that is isolated early in hospitalization [20] [21]. Furthermore, a recent report named *Acinetobacter baumannii* as the most prevalent organism in a tropical burn unit [22]. We also found that *Acinetobacter* became the most prevalent isolate in the last three years of the study and the fourth most common isolate overall. *Acinetobacter* has become resistant to most common antibiotics, also referred to as multi-drug resistant (MDR). This has a negative impact on burn patients as treating infection and septicaemia is very difficult in the presence of MDR *Acinetobacter*. Empirical antibiotic therapy should consider the growing prevalence of such MDR organisms.

There is a global rise in antibiotic resistance [23]-[26]. In our burns unit, we found that *Acinetobacter* was resistant to almost all of the antibiotics available except colistin. According to some reports, *Acinetobacter* demonstrated high sensitivity to carbapenems [9] [27]. Such was the case in our unit until 2005, after which the resistance of *Acinetobacter* to carbapenems increased to more than 90%.

Another organism isolated increasingly is *K. pneumoniae*. Unfortunately, it shows increasing resistance to most antibiotics now, including the carbapenems. In the US, *K. pneumoniae* resistance to carbapenems began in late 1990s [28]; in our unit, the resistance appeared in 2012.

Bacterial sensitivity to antibiotics varies according to the organism, the antibiotic used and time. MRSA was reported by some centres to be resistant to vancomycin [25] [29]. Fortunately, MRSA susceptibility to vancomycin remained at nearly 100% in our unit. In fact, MRSA demonstrated increasing susceptibility to many antibiotics over the study period.

We also found increasing susceptibility to antibiotics in other organisms like *Klebsiella* species, and *P. aeruginosa*. Similar findings regarding *Pseudomonas aeruginosa* sensitivity were reported previously by other centres [26]. On the other hand, other investigators found increasing resistance of *P. aeruginosa* to antibiotics [13]. In our unit, MDR *Pseudomonas aeruginosa* is still a rare occurrence.

The infection control measures taken by our hospital, and the burns unit in particular, to control MDR *Acinetobacter* and MRSA are: strict hand hygiene; screening of the patients at the referring hospital and after arrival to the burns unit; strict isolation of colonized and infected cases; protective aprons, masks and gloves when staff are in contact with the patients; restricting the visitors to two per day; and regular auditing and rounds by the infection control nurse.

Despite infection control measures, the control of MDR *Acinetobacter* is difficult. Burns patients require pro-

longed hospital stays and some require prolonged ventilation as well as indwelling lines and catheters. The immunity of these patients is depressed and their wound recovery is slow. All of these factors increase the risk of infection. As might be expected, the majority of mortalities in our unit have MDR *Acinetobacter* growth.

The rise in MDR organisms is a global phenomenon. There is a need for new antibiotics to control such organisms. We observed in this review that microorganisms were sensitive to antibiotics that have been out of use for a long time.

## 5. Conclusion

In our hospital burns units, *P. aeruginosa* remains the most commonly isolated organism overall, followed by *S. aureus* and MRSA. Multidrug resistant *Acinetobacter* prevalence is increasing and colistin remains the only antibiotic that is effective against it. Enterobacteria, carbapenem-resistant, were isolated occasionally suggesting their growing prevalence. Although certain organisms demonstrate increasing resistance to antibiotics, other organisms display increasing sensitivity. MRSA is highly sensitive to vancomycin and shows a progressive rise in sensitivity to a number of antibiotics.

## Conflict of Interest

All authors declare that they have no conflict of interest.

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