Nature of bacteria found on some wards in Sultan Qaboos University Hospital, Oman

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ABSTRACT

This study aims to determine what objects lying in the hospital environment or brought in from outside contribute to the introduction of bacteria associated with nosocomial infections. One hundred swab specimens collected from children's toys, sinks, door handles, telephone handsets and flowers brought into the hospital were plated on different culture media. Colonial growth on the media was purified and identified subsequently using standard bacteriological methods. Of the 100 samples cultured, 61 (61%) grew a range of bacteria including *Pseudomonas aeruginosa* (n=14, 23.0%), *Acinetobacter* spp. (n=13, 21.3%), *Serratia* spp. (n=9, 14.7%), *Staphylococcus epidermidis* (n=9, 14.7%), *Stenotrophomonas maltophilia* (n=4, 6.6%), *Staphylococcus aureus* (n=4, 6.6%), *Enterobacter cloacae* (n=3, 4.9%), *Pantoea* sp. (n=2, 3.3%), *Chryseobacterium* sp. (n=2, 3.3%) and *Klebsiella pneumoniae* (n=1, 1.6%). Although all the *Serratia, Enterobacter, Klebsiella* and *Pantoea* species isolates showed varying degrees of resistance to gentamicin, ceftriaxone, cefuroxime and cefotaxime, all were resistant to ampicillin. *Chryseobacterium* and *Stenotrophomonas* species isolates were resistant to amikacin, imipenem, gentamicin and ceftazidime, to which only three isolates of *Pseudomonas* species were resistant. All the staphylococcal isolates were susceptible to methicillin. Although there has been no major outbreak of a nosocomial infection in the hospital, it is strongly recommended that effective control measures (e.g., sampling the hospital water supply, disinfecting children's toys, use of appropriate hand washing and checking some of the disinfectants for presence of bacteria) are needed. These measures are necessary to ensure that the antibiotic-resistant strains identified in this study are not allowed to spread in the hospital.

KEY WORDS: Antibiotics.
Cross infection.
Hospitals.
Infection control.

Introduction

Hospitals are high-risk environments in which in-patients often succumb to infection within 48–72 hours of admission. Such infections are termed nosocomial.[1] Organisms associated with nosocomial infection in hospitals originate from human carriers, tap water and equipment used in the hospital.[2–4]

Person-to-person contact among medical staff and between medical staff and patients appears to be the most common route of transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase-negative, methicillin-resistant staphylococci.[5] Tautman *et al*.[3] suggests hospital water to be the most common source of nosocomial infections due to *Pseudomonas aeruginosa, Serratia marcescens* and *Acinetobacter anitratus*. Simor *et al*.[6] found *P. aeruginosa* to be abundant in hospital sinks, basins and baths, while Anaissie[2] attributed over 1400 deaths that occurred each year in the USA to nosocomial infection caused by *P. aeruginosa*. These deaths were associated with contamination of bedpans, drinking water, respiratory equipment, showers and poor hand washing procedures by hospital staff and patients.

Some workers have found that most stethoscopes used by doctors are contaminated by *Staphylococcus aureus*, coagulase-negative staphylococci and in some cases by *Bacillus, Candida* and *Aspergillus* species.[7] Gloves have been found to be reservoirs for *Acinetobacter anitratus*, while 75% of nosocomial infections occur in surgical patients and are due to endogenous flora.[4,8]

The present study aims to determine what items present on some wards at Sultan Qaboos University Hospital (SQUH) serve as receptacles for bacteria associated with nosocomial infection, the extent to which some items introduced onto the wards from outside harbour bacteria associated with nosocomial infections, and the antibiotic sensitivity pattern of the organisms isolated.

It is hoped that the information obtained will gauge the magnitude of the problem and thus contribute to improvements in infection control processes in the hospital.

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Materials and methods

Children's, female medical and gynaecology wards at SQUH were selected for investigation and items sampled on the wards included children's toys, sinks, door handles, telephone handsets and flowers brought in by visitors.

Swabbing of items and media inoculation

Each object was swabbed with a sterile swab moistened with

sterile physiological saline. Swabs were broken into trypticase soy broth and incubated for 6 h at 37°C. Thereafter, each was plated on blood agar (BA; Oxoid, UK), cysteine lactose electrolyte-deficient (CLED; Oxoid) and mannitol salt agar (MSA; Oxoid). Plates were incubated for 48 h at 37°C, while trypticase soy broth was re-incubated for a further 18 h and subsequent growth cultured on BA, MSA and CLED media. Growth was identified using appropriate bacteriological procedures[9] and API 20 systems (bioMérieux, France).

Table 1. Types of objects sampled from each ward and bacteria isolated.

Ward	Object	Samples	Bacteria isolated	No. of isolates
CH4	Toys	34	*P. aeruginosa*	2
			S. epidermidis	4
			Acinetobacter spp.	5
			S. plymuthica	2
			S. rubidaea	1
			S. maltophilia	2
			P. agglomerans	2
	Sinks	4	*P. aeruginosa*	3
			S. liquefaciens	1
	Door handles	10	*P. aeruginosa*	4
			Acinetobacter spp.	2
	Telephones	2	*S. aureus*	1
GYNI	Flowers	10	*P. aeruginosa*	2
			S. epidermidis	1
			Acinetobacter spp.	3
			K. pneumoniae	1
	Sinks	6	*P. aeruginosa*	1
			Acinetobacter sp.	1
			S. marcescens	1
			S. liquefaciens	1
			C. meningosepticum	1
			C. indologenes	1
	Door handles	6	*S. aureus*	1
			Acinetobacter spp.	2
			S. maltophilia	1
	Telephones	5	*S. aureus*	1
			S. marcescens	1
			S. maltophilia	1
FMW1	Flowers	4	*P. aeruginosa*	1
			S. epidermidis	1
			E. cloacae	2
	Sinks	5	*P. aeruginosa*	1
			S. marcescens	2
	Door handles	6	*S. epidermidis*	3
	Telephones	8	*S. aureus*	1
			E. cloacae	1
Total		100		61

CH4: Children's Ward 4,
GYN1: Gynaecological Neonatal Ward 1,
FMW1: Female Medical Ward 1.

Sensitivity determination

Each isolate, grown overnight in brain heart infusion, was standardised to contain 1 x 10^4 colony-forming units (CFUs)/mL for Gram-negative rods and 1 x 10^5 CFUs/mL for Gram-positive cocci using McFarland's Standard Opacity Tube 0.5.[9] The sensitivity pattern of each standardised isolate was then determined on diagnostic sensitivity test agar (DST; Oxoid) using Stokes' method[10] and appropriate antibiotics and control organisms (*S. aureus* NCTC 6571 for staphylococcal isolates, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662 for enterobacteria and oxidase-positive isolates, respectively).[9]

Results

Table 1 shows the types of bacteria isolated from different objects on the three wards, while Table 2 shows the percentage distribution of each isolate. *Pseudomonas aeruginosa* (n=14, 23.0%) was the most common organism, followed by *Acinetobacter* spp. (n=13, 21.3%), *S. epidermidis* (n=9, 14.7%), *Serratia marcescens* (n=4, 6.5%), *Staphylococcus aureus* (n=4, 6.6%), *Stenotrophomonas maltophilia* (n=4, 6.6%), *Enterobacter cloacae* (n=3, 4.9%), *Serratia plymuthica*, *S. liquefaciens* and *Pantoea agglomerans* (n=2 [3.3%], respectively), and one each of *S. rubidea, Klebsiella pneumoniae, Chryseobacterium meningosepticum* and *C. indologenes* (1.6%).

The resistant pattern of the isolates is presented in Table 3. Besides *Acinetobacter* spp., which showed 10 (77%) isolates resistant to ampicillin, all the other enterobacterial isolates were resistant to this antibiotic. However, *Enterobacter* spp. were resistant to gentamicin, ceforuxime and co-trimoxazole while *Klebsiella* spp. were resistant only to gentamicin.

Although *Pseudomonas aeruginosa* showed varying resistance to amikacin, imipenem and gentamicin, all the *Chryseobacterium* and *Stenotrophomonas* species isolates were resistant to the antibiotics. In contrast, all the *Stenotrophomonas* species isolates were sensitive to co-trimoxazole (not shown in the table).

Table 2. Percentage ( ) distribution of isolates according to ward.

Ward	CH4	GYN1	FWM1	Total
	28	21	12	61
P. aeruginosa	9	3	2	14 (23.0)
Acinetobacter species	7	6	0	13 (21.3)
S. aureus	1	2	1	4 (6.6)
S. epidermidis	4	1	4	9 (14.7)
Serratia species	4	3	2	9 (14.7)
Pantoea species	2	0	0	2 (3.3)
S. maltophilia	2	2	0	4 (6.6)
E. cloacae	0	0	3	3 (4.9)
K. pneumoniae	0	1	0	1 (1.6)
Chryseobacterium species	0	2	0	2 (3.3)

Discussion

Many types of bacteria and fungi are associated with nosocomial infections.[7,11] The sources of these organisms in most cases originate from objects found in the hospital environment.[2,3,6,7,12] Trautman *et al*.[3] found contaminated tap water to be a reservoir of nosocomial pathogens as *P. aeruginosa, S. maltophilia* and other bacteria of faecal and plant origin were present.

In the present study, *P. aeruginosa* was more prevalent than other organisms and was found mostly on toys and on the sinks used by hospital staff and patients (Tables 1 and 2). The presence of *P. aeruginosa* on objects used regularly by children is not encouraging, as this organism is a highly opportunistic pathogen associated with infant pneumonia and bacteraemia.[2,11] Although sinks and toys are disinfected from time to time, *P. aeruginosa* is known to withstand the sterilising effect of disinfectants.[13] The toys were disinfected after the children had used them and swab samples were taken as the children were playing with them. Thus, it is likely that the organism was not killed by the disinfectant used or that the toys were recontaminated by the children or by the receptacles used to store the toys after disinfection.

Anaissie *et al*.[2] found that infection control measures alone, even though very important in the hospital, were insufficient to prevent nosocomial infections due to pseudomonads and other waterborne pathogens. Therefore, they recommended heightened surveillance for organisms associated with nosocomial infections.

In addition to the presence of waterborne microbial pathogens on the toys, bacteria of faecal origin (e.g., *Serratia* and *Acinetobacter* species) were also present, which suggests inadequate hand washing by the medical staff or by the children who use them or by the parents who assist their children when playing with the toys.

Pantoea agglomerans, although an opportunistic pathogen, is primarily of plant origin but can be found in the saliva of alligators and occasionally in the faeces of humans.[14] The organism is associated with bacteraemia, endocarditis and wound infections.[14,15] Its presence on toys indicates that some organisms are brought in from the community to the hospital environment.

The presence of *Chryseobacterium meningosepticum* and *C. indologenes* on the surface of sinks suggests that cleaning and disinfection of sinks after use is not effective, that the tap water used in the hospital may not be effectively chlorinated or filtered or that there are leaks in the water distribution system. Anassie *et al*.[2] recommended the use of hot water for rinsing most hospital equipment after cleaning.

The spread of *Serratia marcescens* in some hospitals was traced to contaminated washing solutions. Bosi *et al*.[16] found *S. marcescens* on surfaces where chlorhexidine disinfectant was used for cleaning sinks. As sinks are used for cleaning or rinsing dirty articles, such articles can be re-contaminated by the sinks.

All the bacteria isolated in this investigation are known to survive on wet surfaces for a very long time and this may explain their occurrence in such areas as sinks, door handles, toys and telephone hand sets.[9,17] *Chryseobacterium* spp. were associated with neonatal and non-neonatal septicaemia and meningitis.[18–20]

In this study, it was observed that flowers that visitors to the hospital brought in harbour *Acinetobacter, Klebsiella* and *Enterobacter* species in addition to *Pseudomonas aeruginosa* (Table 1). The sources of contamination may be due to the water used for watering them during culture or for keeping them alive during their stay with the patient. It is likely that such items can serve as reservoirs from which to contaminate the wards.

The resistant pattern of the isolates in this study conforms to the antibiotic pattern of the clinical isolates in the hospital, which has suffered no major outbreak of nosocomial infection. The *Pseudomonas* spp. isolates contained only two strains that were resistant to imipenem and ciprofloxacin. These two strains may have been hospital-acquired, although no further investigation was conducted.

Studies have shown that *Stenotrophomonas* spp. are strong

Table 3. Antibiotic resistant patterns (%).

Organisms	Total isolates	Antibiotic resistant patterns					
		AK	IMI	PIP	CAZ	CN	CIP
P. aeruginosa	14	3 (21)	2 (14)	1 (7)	3 (21)	4 (27)	2 (14)
Chryseobacterium spp.	2	2 (100)	2 (100)	2 (Nil)	2 (100)	2 (100)	2 (Nil)
Stenotrophomonas spp.	4	4 (100)	4 (100)	4 (100)	1 (25)	4 (100)	4 (Nil)
		CN	CRO	CXM	AMP	SXT	CTX
Acinetobacter spp.	13	3 (23)	3 (23)	7 (54)	10 (77)	3 (23)	4 (31)
Serratia spp.	9	2 (22)	2 (22)	7 (77)	9 (100)	3 (33)	2 (22)
Enterobacter spp.	3	3 (100)	2 (67)	3 (100)	3 (100)	3 (100)	2 (67)
Klebsiella sp.	1	1 (100)	1 (Nil)	1 (Nil)	1 (100)	1 (Nil)	1 (Nil)
Pantoea spp.	2	1 (50)	2 (100)	1 (50)	2 (100)	2 (Nil)	2 (100)
		MET	SXT	CN	FD	P	E
S. aureus	4	4 (Nil)	1 (25)	2 (50)	4 (Nil)	3 (75)	2 (50)

Nil: no resistance (sensitive), AK: amikacin, AMP: ampicillin, CAZ: ceftazidime, CIP: ciprofloxacin, CRO: ceftriaxone, CN: gentamicin, CTX: cefotaxime, CXM: cefuroxime, E: erythromycin, FD: fusidic acid, IMI: imipenem, MET: methicillin, P: penicillin, PIP: piperacillin, SXT: co-trimoxazole.

β-lactamase producers.[9,17] This is confirmed by the findings of the present study, as the organism was resistant to imipenem and piperacillin. Other Gram-negative rod isolates showed variable resistance to gentamicin, ceftriaxone, cefuroxime, co-trimoxazole and cefotaxime (Table 3). However, the highest resistance was noted with ampicillin, while three strains of *Acinetobacter* spp. and one strain of *Pantoea* sp. were sensitive to this antibiotic.

Husni *et al*.[21] found *Acinetobacter* spp. involved in nosocomial infection to be multidrug resistant. This is in contrast to the three *Acinetobacter* spp. encountered in the present study. *Staphylococcus* spp. contained no methicillin-resistant strains, suggesting that they were not hospital-acquired strains, which are usually resistant to methicillin (Table 3);[9,17] however, more work is necessary to confirm this observation.

Pen-Yi *et al*.[18] and Bloch *et al*.[19] found isolates of *Chryseobacterium* spp. to be sensitive to ciprofloxacin but resistant to most β-lactamase inhibitors including imipenem. The present study demonstrates isolates to be sensitive to piperacillin and ciprofloxacin but resistant to imipenem, ceftazidime, gentamicin and amikacin.

Conclusions

This study demonstrates that children's toys, hospital sinks, telephone hand sets, door handles and flowers brought from outside serve as reservoirs for some bacteria found on hospital wards. Some of these organisms (e.g., *Pseudomonas, Stenotrophomonas* and *Chryseobacterium* species) are known to be waterborne pathogens. Organisms of faecal/plant origin (e.g., *Acinetobacter, Klebsiella, Staphylococcus, Enterobacter, Serratia* and *Pantoea* species) were also isolated.

Organisms such as *Pantoea, Chryseobacterium* and *Stenotrophomonas* species are emerging opportunistic pathogens that should not be found readily on wards because they are strong β-lactamase producers and the drugs for their treatment are usually costly. In addition to *Stenotrophomonas* and *Chryseobacterium* species, which show multiple resistance to some antibiotics, other strains encountered in this study show varying resistances to the antibiotics used in their antibiograms.

It is strongly recommended that effective control measures be implemented to check the spread of some of the organisms by sampling the hospital water supply, disinfecting children's toys thoroughly, appropriate hand washing and checking some of the washing solutions for the presence of organisms associated with nosocomial infection. □

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References

1 Bennet JV, Brachman PS. *Hospital infections* (3rd edn). Boston: Little Brown, 1992: 3–19.
2 Anaissie EJ, Penzak SR, Dignani C. The hospital water supply as a source of nosocomial infections: a plea for action. *Arch Intern Med* 2002; **162:** 1483–92.
3 Trautmann M, Michaisky H, Wiedeck H, Radosavljevic V, Ruhnke M. Tap water colonization with *Pseudomonas aeruginosa* in a surgical intensive care unit (ICU) and relation to Pseudomonas infection of ICU patients. *Infect Control Hosp Epidemiol* 2001; **22:** 49–52.
4 Leaper DJ. Risk factors for surgical infection. *J Hosp Infect* 1999; **30:** 127–39.
5 Oie S, Kamiya A. Survival of methicillin-resistant *Staphylococcus aureus* (MRSA) on naturally contaminated dry mops. *J Hosp Infect* 1996; **34:** 145–9.
6 Simor AE, Ramage L, Wilcox L, Bull SB, Bialkowska-Hobrzanska H. Molecular and epidemiological study of multiresistant *Serratia marcescens* infections in a spinal cord injury rehabilitation unit. *Infect Control* 1988; **9:** 20–7.
7 Francis Marie AP, Emmanuel Edwin RD, Remedios FC. Stethoscopes: a potential source of nosocomial infections. *Phil J Microbiol Infect Dis* 200; **29** (2): 9–13.
8 Patterson JE, Ueechio J, Pantelick EL. Association of contaminated gloves with transmission of *Acinetobacter calcoacticus* var *anitratus* in an intensive care unit. *Am J Med* 1991; **91:** 479–83.
9 Collee JG, Fraser AG, Marmion BP, Simmons A. *Mackie and McCartney's practical medical microbiology* (14th edn). Edinburgh: Churchill Livingstone, 1996.
10 Stokes EJ, Ridway GI, Wren MWD. *Clinical microbiology* (7th edn). London: Arnold, 1993: 248.
11 Richards MJ, Edwards JR, Culver DU, Gaynes RP and the National Nosocomial Infections Surveillance System. Nosocomial infections in paediatric intensive care units in the United States. *Paedia* 1999; **103** (4): 1–7.
12 Livornese LL, Dias S, Samel C *et al*. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. *Ann Intern Med* 1992; **117:** 112–6.
13 Foca M, Jakob K, Whittier S *et al*. Endemic *Psseudomonas aeruginosa* infection in a neonatal intensive care unit. *N Engl J Med* 200; **343:** 695–700.
14 Olenginski TP, Bush DC, Harrington TM. Plant thorn synovitis: an uncommon cause of monoarthritis. *Smi Arthritis Rheum* 1991; **21:** 40–6.
15 Flandry F, Lisecki EJ, Dominque GJ, Nichols Rddad RJ. Initial antibiotic therapy for alligator bites: characterization of the oral flora of alligator (*Mississipiensis*). *South Med J* 1989; **82:** 282–6.
16 Bosi CA, Ragli-Davin, Charrel R, Rocca B, Monnet D, Bellet C. *Serratia marcescens* nosocomial outbreak due to contamination of chlorhexidine solution. *J Hosp Infect* 1996; **33:** 217–24.
17 Timbury MC, McCartney AC, Thakker B, Ward KN. *Notes on medical microbiology* Edinburgh: Churchill Livingstone, 2002.
18 Lin PY, Chu C, Su LH, Huang CT, Chang WY, Chiu CH. Clinical and microbiological analysis of bloodstream infections caused by *Chryseobacterium meningosepticum* in non-neonatal patients. *J Clin Microbiol* 2004; **42** (7): 3353–5.
19 Bloch KC, Nadarajah R, Jacobs R. *Chryseobacterium meningosepticum*: an emerging pathogen among immunocompromised adults. *Medicine* 1997; **76:** 30–41.
20 Chiu CH, Waddingdon M, Hsieh WS, Greenberg D, Schreckenberger PC, Carnahan AM. Atypical *Chryseobacterium meningosepticum* and meningitis and sepsis in newborn and the immunocompromised. *Taiwan Emerg Infect Dis* 200; **6:** 481–6.
21 Husni RN, Goldstein LS, Arroliga AC *et al*. Risk factor for outbreak of multi-drug resistant *Acinetobacter* nosocomial pneumonia among intubated patients. *Chest* 1999; **115:** 1378–82.