



Microbial Assessment of Swimming Pools from Selected Hotels in Osogbo Metropolis, Osun State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FAA designed the study, performed the statistical analysis and wrote the protocol. Author OAT managed the analyses of the study, the literature searches and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The presence of microorganisms in swimming pool water is of concern today and health threat because they have been shown to cause infections especially gastroenteritis infections with probable transfer of these organisms from swimmers into the swimming pool. The microbiological qualities of five swimming pools in Osogbo was studied for the wet and dry seasons. Bacterial isolates were identified based on morphological and biochemical methods with the aid of Bergey's Manual of Determinative Bacteriology. The Fungal isolates were identified using lactophenol cotton blue method. The heterotrophic bacterial population count in swimming pool water samples ranged from 1.7×10^2 cfu/ml to 3.6×10^2 cfu/ml to 5.2×10^2 cfu/ml. A total of 43 bacterial species which belonged to seven genera, and comprising *Pseudomonas* sp, *Klebsiella* sp, *Aeromonas* sp, *Citrobacter* sp, *Shigella* sp, *Proteus* sp and *Staphylococcus aureus* were isolated. The fungal

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population during the study ranged between 1.2×10^2 cfu/ml and 3.9×10^2 cfu/ml. Twenty five (25) fungi species which belonged to ten genera including *Absidia cylindrospora*, *Aspergillus niger*, *Aspergillus repens*, *Cephalosporium corda* *Cladosporium*, *Fusarium* sp, *Monotospora altissima*, *Penicillium citrinum*, *Schizophyta minuta* and *Trichophyton* sp were isolated. The coliforms isolated were *Shigella dysenteriae*, *Shigella paradysenteriae*, *Shigella sonnei* and *Citrobacter freundii*. The isolates were resistant to augmentin (95.3%), cotrimoxazole (93.7%), amoxicillin and nitrofurantoin (83.7%), tetracycline (72.1%), gentamicin (62.8%) and ofloxacin, (0%). The study concluded that swimming pools are contaminated with different microbial organisms of potential diseases with capable of causing diseases/infections or potentially pathogenic to man, and this requires adequate care and proper monitoring of swimming pools.

Keywords: Microbial assessment; swimming pools; bacteria; fungi; Osogbo Metropolis.

1. INTRODUCTION

A variety of microorganisms can be found in swimming pools and similar recreational water environments, which may be introduced in a number of ways. Infected users can directly contaminate pool waters and the surfaces of objects or materials at a facility with pathogens (Fungi), which may lead to skin infections in other persons who come in contact with the contaminated water or surfaces [1,2]. Although gastrointestinal disease is the most common disease encountered, skin, eye and neurological symptoms have also been related to recreational and environmental water contact [3]. Opportunistic pathogens can also be shed from users and transmitted through surfaces and contaminated water. Some bacteria, most notably non- faecally-derived bacteria, may accumulate into biofilms and present an infection hazard [4]. In addition, certain free living aquatic bacteria and amoebae can grow in pool.

Bacterial pathogens causing mainly gastrointestinal disease include *Shigella* species, *Salmonella* species, *Campylobacter* species, and *Escherichia coli* (for example, *Escherichia coli* O157:H7). *Leptospira* species are present in the environment and humans become infected through cuts to the skin or via mucous membranes [5]. Protozoa that cause gastrointestinal disease include *Cryptosporidium parvum* and *Giardia duodenalis*. Other parasites include amoebae such as *Acanthamoeba* species, *Hartmanella vermiformis* and *Naegleria fowleri*. Gastrointestinal symptoms are also caused by small round structured viruses (SRSV), astroviruses, caliciviruses, and rotaviruses. The SRSV appear to be the most important cause of viral gastro intestinal disease associated with recreational water contact [5]. Other human enteric viruses found in surface waters used for recreation, include hepatitis A

which causes disease of the liver. However, the enteroviruses and culturable adenoviruses have, to date, not been linked to specific symptoms. Properly managed and disinfected swimming pool waters, spa waters and other pool waters should not contain viable micro-organisms.

However, *Cryptosporidium* and *Giardia* are of concern since they are more resistant to disinfection than other micro-organisms referred to above. In addition, *Pseudomonas aeruginosa* has been associated with eye, skin and ear infections after contact with spa pool waters or hot-tub waters [4]. Cases of pneumonia and urinary tract infections have also been reported following the use of contaminated pools [6,7]. Also, *Mycobacterium marinum* has been associated with skin infections and pool water contact, and adenovirus conjunctivitis has been linked with swimming pool water [8]. The abrasive non-slip flooring that often surrounds pool waters has been associated with *Trichophyton* (athletes foot) and the wart virus [9]. Nigeria has no swimming pool water standard but has adopted that of the [1,10].

However, there is a sparse of information on microbial quality of swimming pools from hotels in Osogbo Metropolis, Southwest, Nigeria. The present study was carried out to isolate and determine the antimicrobial resistance of the microbial isolates of the pools with a view to determining the water quality safety.

2. MATERIALS AND METHODS

2.1 Estimation of Bacterial and Fungal Isolates Population

Microbial analysis of water samples was determined by plate count methods. Nutrient Agar, Sabouraud Dextrose Agar, MacConkey broth were used for the isolation of

microorganisms present in the swimming pools by using standard microbiological methods [11]. The bacterial plates and fungal plates were incubated at 37°C for 24 h and 25°C for 72 h respectively. Multiple tube technique of three tubes of 10 ml double strength, as well as three tubes of 1 ml and 0.1 ml single strength were used to determine the presence or absence of coliform and *Escherichia coli* count using MacConkey broth and Eosin Methylene Blue (EMB) agar respectively. Isolation and identification of bacteria in water samples were carried out by using cultural, morphological and biochemical tests [12].

2.2 Characterization of Bacterial and Fungal Isolates

The representative bacterial and fungal colonies on each plate were isolated and sub-cultured to obtain pure colonies. The pure bacterial and fungal isolates were maintained on nutrient agar and sabouraud dextrose agar slants respectively, and were then stored in the refrigerator at 4°C. The presumptive identification of bacterial isolates was done after the determination of their biochemical tests using Bergey's Manual of Determinative Bacteriology [13].

2.3 Cultural Characteristics

The cultural characteristics and the total number of colonies on agar plate were recorded. The total colony counts were multiplied with the dilution factor [14], microbial counts were expressed as colony forming units per ml (cfu/ml) of water samples. The colonial appearance of each bacterial isolate on the plate was observed and classified based on the following characteristics; colour, shape, edge, elevation, surface and opacity [15].

2.4 Biochemical Identification of the Isolates

Biochemical tests were carried out for the identification of bacterial isolates. These includes catalase test, coagulase test, methyl red, Voges-Proskauer test, oxidation/fermentation test, nitrate reduction test, sugar fermentation (glucose, sucrose, maltose, mannitol, lactose) test, reaction on sulphide indole motility medium. These biochemical tests were carried out according to Dubey and Maheshwari [12], 18 to 24 h broth culture of each isolate were used. An

un-inoculated sterile medium was used as control in all the tests carried out.

2.5 Identification of Fungal Isolates

Identification of fungi was done by examining the fungi under the microscope, with reference to Barnett and Hunter [16]. Seven days pure culture of fungi was used for the microscopic identification. A wet mount of the culture was prepared on a clean slide by adding a drop of cotton blue lactophenol reagent. The slide was then covered with a cover slip and viewed under the microscope at the X40 objective lens.

3. RESULTS AND DISCUSSION

In Table 1, a total of 43 bacterial species which belonged to seven genera were isolated, comprising *Pseudomonas* sp, *Klebsiella* sp., *Citrobacter* sp., *Proteus* sp, *Aeromonas* sp. *Shigella* sp and *Staphylococcus* sp. It was observed that *Aeromonas* sp., *Klebsiella* sp., *Citrobacter* sp., *Pseudomonas* sp. and *Staphylococcus* sp. were frequently isolated from the water samples. This observation was supported by the report of Ali- Shtayeh et al. [17].

Various species of *Klebsiella* sp were the most commonly isolated contaminants from the swimming pool water. The presence of these organisms in swimming pool water is of health concern because they have been shown to cause infections especially gastroenteritis infections with probable transfer of these organisms from swimmers into the swimming pool. These organisms have been shown as potential contaminants of swimming pool water [18].

Some of the enteric bacteria encountered in this study are pathogenic to human being; some are non-pathogenic, while some are opportunistic pathogens of man and have been associated with nosocomial infections [19,20]. The high level of contamination of swimming pool water was a potential threat to man because of the fact that water is necessary for the existence of man, hence there is possibility of bacteria being transmitted. In addition, the relative abundance of the resident or normal skin flora, as well as transient bacteria that may be found on the skin could enhance an easy transfer to other swimmers through the swimming pool water [21].

Table 1. Frequency and percentage distribution of the bacteria Isolated from the swimming pool water samples

Probable bacteria	Frequency	Percentage occurrence (%)
<i>Aeromonas hydrophila</i>	7	16.3
<i>Citrobacter freundii</i>	5	11.6
<i>Klebsiella edwardsii</i>	4	9.3
<i>Klebsiella pneumoniae</i>	5	11.6
<i>Klebsiella oxytoca</i>	3	6.9
<i>Proteus rettgeri</i>	2	4.7
<i>Pseudomonas aeruginosa</i>	6	14
<i>Pseudomonas alkaligenes</i>	4	9.3
<i>Shigella dysenteriae</i>	1	2.3
<i>Shigella paradysenteriae</i>	1	2.3
<i>Shigella sonnei</i>	2	4.6
<i>Staphylococcus aureus</i>	3	7
Total	43	100

Pseudomonas aeruginosa can cause a range of infections but rarely causes serious illness in healthy individuals without some predisposing factors. It predominantly colonizes damaged sites such as burn and surgical wounds, the respiratory tract of people with underlying disease and physically damaged eyes. From these sites, it may invade the body, causing destructive lesions or septicaemia and meningitis [22].

Staphylococcus aureus is the most common cause of staphylococcus infections. It is a spherical bacterium, frequently found in the nose and skin of a person. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils, cellulitis, folliculitis, scalded skin syndrome and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, and septicaemia. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections, [23]. Bacteria such as *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staph. aureus* were detected from the swimming pool [24]. These microorganisms were also detected from swimming pools in Lagos, Ilorin and South-South zone of South-eastern Nigeria [25,26].

Other microorganisms with high incidences such as *Aeromonas hydrophila* and *Citrobacter freundii* have been shown to cause infection and they have frequently been isolated from the water samples (Table 1). *Aeromonas hydrophila* is a free living microorganism, associated with gastroenteritis while *Citrobacter freundii* is an opportunistic

microorganism associated with urinary tract infection [4].

Shigella dysenteriae, *Shigella paradysenteriae*, *Shigella sonnei* and *Proteus rettgeri* are the least occurring organisms as contaminants in the present study (Tables 1 and 2), the presence of these organisms in swimming pool water is a cause for alarm because they have been shown to have the potential to cause infections especially diarrheal disease, fever and chest pain with probable transfer of these organisms from the swimmers into the swimming pool water [27].

In Table 3, a total of 25 fungal species which belonged to ten genera were isolated from the swimming pool water samples. The highest fungal population was recorded in rainy season for Pool A, Pool C and Pool E, while the lowest population was recorded in Pool D and Pool B. Pool E had the highest fungal population among the other swimming pools and this may be attributed to maintenance failures.

The isolated fungi were *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Cladosporium* sp., *Scizophyta* sp., *Absidia* sp., *Trichophyton* sp., *Cephalosporium* sp., and *Monotospora* sp. *Trichophyton* sp are associated with skin diseases affecting millions of people worldwide. *Fusarium* sp can cause mycotoxicosis in human, and localized infections such as arthritis and cystitis. *Cladosporium* is associated with eye and skin infection [28]. The uncommon fungi such as *Monotospora altissima* and *Cephalosporium corda* isolated from swimming pool water samples may be attributed to the moist environment of the swimming pools which favours the growth of the fungi.

Table 2. Biochemical characteristics of bacterial isolates from swimming pool water samples

Isolate Code	Morphology					Sugar fermentation										O-F	Nitrate Reduction	Probable Identity
	Gram's reaction	Spore production	Catalase	Coagulase	Oxidase	Reaction TSI	Reaction SIM	Utilization citrate	MR	VP	Glucose	Maltose	Mannitol	Sucrose	Lactose			
1A ₁	-	-	+	-	-	++-	+++	+	+	-	+	+	+	+	+	F	+	<i>Citrobacter freundii</i>
1B ₃	-	-	+	-	+	+-	+++	-	+	-	+	-	-	-	-	F	+	<i>Klebsiella pneumoniae</i>
1B ₄	-	-	+	-	+	---	+++	-	-	-	-	-	-	-	-	-	+	<i>Pseudomonas alkaligenes</i>
1D ₁	-	-	+	-	+	+-	+++	+	+	+	+	+	+	+	+	F	+	<i>Klebsiella oxytoca</i>
1D ₂	-	-	+	-	+	+-	+++	-	+	-	+	-	+	+	-	F	+	<i>Aeromonas hydrophilia</i>
1E ₁	-	-	+	-	+	+-	+++	+	+	+	+	+	+	+	-	F	+	<i>Proteus rettgeri</i>
6A ₁	-	-	+	-	-	+-	+++	-	+	-	+	-	-	-	-	F	+	<i>Shigella dysenteriae</i>
6B ₂	-	-	+	-	+	+-	+++	-	+	-	+	+	+	+	-	F	+	<i>Klebsiella edwardsii</i>
6C ₃	-	-	+	-	+	+-	+++	-	+	+	+	+	+	+	-	F	+	<i>Klebsiella edwardsii</i>
6E	+	-	+	+	-	+-	+++	-	+	-	+	+	+	+	+	F	+	<i>Staphylococcus auerus</i>
6E ₁	-	-	+	-	+	---	+++	-	-	-	-	-	-	-	-	None	+	<i>Pseudomonas aeruginosa</i>
7A ₁	-	-	-	-	+	+-	+++	-	+	-	+	+	+	+	-	F	+	<i>Shigella sonnei</i>
7D ₁	-	-	+	-	+	+-	+++	-	-	-	+	+	+	-	-	F	+	<i>Shigella paradysenteriae</i>
7E ₄	-	-	-	-	+	---	+++	-	-	-	-	-	-	-	-	None	+	<i>Pseudomonas aeruginosa</i>
7E ₆	-	-	+	-	-	+-	+++	-	+	-	+	-	+	+	-	F	+	<i>Aeromonas hydrophilia</i>

Legend: F - Fermentative + Positive; O – Oxidative - Negativ

The most frequently isolated of these fungi species were *Aspergillus*, *Monotospora* and *Cladosporium*. The result above was similar to the previous work that reported similar fungal species from the water of swimming pools in a survey in Nigeria [29]. The mean coliform count of water samples from swimming pools A-E were above the WHO Guidelines limit of <1 cfu/100 ml. The high values of coliforms signified contamination [29].

Infections with antibiotic resistant bacteria have negative impacts on public health due to an incidence of treatment failure and severity of disease [30]. Treatment of infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics [31].

The incidence of multiple antibiotic resistances of the bacteria revealed from this study (Tables 4 and 5) that *Pseudomonas aeruginosa* showed 100% multi-drug resistance. Among the Gram positive bacteria, only *Staphylococcus aureus* showed multiple antibiotics resistance (100%) to ampiclox, septrin, and erythromycin respectively. *Staph. aureus* is usually contributed by bathers in the swimming pools [24].

Multiple resistances to antibiotics showed that diseases and illnesses caused by these bacteria cannot be treated by these antibiotics. This can be because of several human activities such as wide spread misuse and abuse of these drugs, and exposure to different antibiotics over time which may induce resistance in the bacterial isolates [27].

Gram negative isolates were resistant to one or more of the antibiotic tested. The relatively high resistance of bacterial pathogens to antimicrobial agents in the current study agreed with the findings of Barat et al. [32] who reported a high frequency of antimicrobial resistance of Gram negative bacteria in swimming pool. The susceptibility of bacterial isolates in swimming pool water varied with antibiotics. The bacterial isolates were 100% sensitive to Ofloxacin. The bacteria were resistant to the augmentin, nitrofuratoin and amoxicillin, with 100% resistance. These antibiotics may represent one of the often prescribed drugs in health care facilities in Nigeria, which has contributed to the abuse of these drugs. The abuse of antimicrobials can result in the development of resistant strains of bacteria in swimming pool

water. The high frequency of resistance by bacteria may result in therapeutic failure in people who have become infected with any of these pathogens. Ofloxacin was very active against all the bacterial isolates. This may be attributed to the fact that the bacteria might not have been able to display any form of resistance to the antibiotic. Also, the drug may be novel to the bacteria during this study. Ofloxacin may therefore be the choice antibiotic for treating infections resulting from the bacteria isolated in this study.

In this study, multi-drug resistance was common among the bacteria isolated from swimming pool water. The results showed that 99.9% of the bacteria were resistant to more than two groups of antibiotics (Tables 6). Resistance to four or more antibiotics was prevalent and this will make the treatment of bacterial infection difficult owing to the high resistance to antibiotics. The majority of antimicrobial resistance which makes treatment of infectious diseases difficult has been attributed to extensive use and misuse of drugs which have favoured the emergence and survival of resistant strains of microorganisms. Infection associated with antibiotic resistant bacteria has negative impacts on public health due to an incidence of treatment failure and severity of disease [30].

Table 3. Frequency and percentage distribution of the Fungi Isolate Isolated in swimming Pool water

Fungal	Frequency	Percentage occurrence (%)
<i>Absidia cylindrospora</i>	2	8.0
<i>Aspergillus niger</i>	3	12.0
<i>Aspergillus repens</i>	3	12.0
<i>Cephalosporium corda</i>	2	8.0
<i>Cladosporium sp</i>	5	20.0
<i>Fusarium sp</i>	1	4.0
<i>Monotospora altissima</i>	5	20.0
<i>Penicillium citrinum</i>	1	4.0
<i>Schizophyta minuta</i>	2	8.0
<i>Trichophyton sp</i>	1	4.0
Total	25	100

Table 4. Percentage antibiotic resistance of gram negative bacteria isolated from swimming pool water samples

Isolated organisms	No of isolate resistance	AMX	TET	AUG	GEN	NAL	NIT	COT	OFL
<i>Aeromonas hydrophilia</i>	7	7 (100)	7 (100)	7 (100)	0 (0.00)	7 (100)	7 (100)	7 (100)	0 (0.00)
<i>Citrobacter freundii</i>	5	5 (100)	5 (100)	5 (100)	5 (100)	0 (0.00)	5 (100)	5 (100)	0 (0.00)
<i>Klebsiella edwardsii</i>	4	4 (100)	0 (0.00)	4 (100)	1 (25.0)	0 (0.00)	4 (100)	4 (100)	0 (0.00)
<i>Klebsiella pneumoniae</i>	5	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	5 (100)	0 (0.00)	0 (0.00)
<i>Klebsiella oxytoca</i>	3	3 (100)	3 (100)	3 (100)	0 (0.00)	3 (100)	3 (100)	3 (100)	0 (0.00)
<i>Proteus rettgeri</i>	2	2 (100)	2 (100)	2 (100)	2 (100)	0 (0.00)	2 (100)	2 (100)	0 (0.00)
<i>Pseudomonas aeruginosa</i>	9	9 (100)	9 (100)	9 (100)	9 (100)	0 (0.00)	9 (100)	2 (100)	0 (0.00)
<i>Pseudomonas alkaligenes</i>	1	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.00)	1 (100)	1 (100)	0 (0.00)
<i>Shigella dysenteriae</i>	1	1 (100)	0 (0.00)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.00)
<i>Shigella paradysenteriae</i>	1	1 (100)	0 (0.00)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0.00)
<i>Shigella sonnei</i>	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (100)	0 (0.00)
Total	40	40 (100)	34 (85)	40 (100)	27 (67.5)	19(47.5)	40(100)	27 (67.5)	0 (0.00)

Key: AMX- Amoxicillin; NAL- Nalidixic acid; TET- Tetracycline; NIT- Nitrofurantoin; AUG- Augmentin;
COT- Cotrimoxazole; GEN- Gentamycin; OFL- Ofloxacin

Table 5. Percentage of Antibiotic resistance pattern of gram-positive bacteria isolated from swimming pool water samples

Organisms	No of Isolate resistance	Disc potency	10 ug	10 ug	30 ug	20 ug	30 ug	25 ug	10 ug	30 ug	30 ug	10 ug
		Antibiotic disc	PEN	GEN	APX	Z	AMX	R	CPX	STR	SXT	E
<i>Staphylococcus aureus</i>	3		0(0.0)	0(0.0)	3(100)	0(0.0)	3(100)	0(0.0)	0(0.0)	0(0.0)	3(100)	(100)

Key: pen – Penicillin; GEN – Gentamycin; APX - Ampiclox ; Z - Zinnacef ; AMX - Amoxicillin; R - Rocephin
CPX – Ciprofloxacin; STR - Streptomycin ; SXT – Septrin; E - Erythromycin

Table 6. Percentage of multiple resistance patterns of gram negative bacteria isolated from swimming pool water samples

Isolates	No of isolates	Resistance pattern	Frequency of occurrence	Percentage (%)
<i>Aeromonas hydrophilia</i>	7	AMX, TET, AUG, NAL, NIT, COT	7	17.5
<i>Citrobacter freundii</i>	5	AMX, TET, GEN, NIT, COT	1	2.5
		AMX, TET, AUG, GEN, NIT, COT	2	5.0
		AMX, TET, AUG, GEN, NAL, NIT, COT	2	5.0
<i>Klebsiella edwardsii</i>	4	AMX, AUG, NIT	2	5.0
		AMX, AUG, NIT, COT	1	2.5
		AMX, AUG, GEN, NIT	1	2.5
<i>Klebsiella pneumoniae</i>	5	AMX, TET, AUG, NAL, NIT, COT	1	2.5
		AMX, TET, GEN, NAL, NIT, COT	1	2.5
		AMX, TET, AUG, GEN, NAL, NIT, COT	2	5.0
		AMX, AUG, NAL, NIT, COT	1	2.5
<i>Klebsiella oxytoca</i>	3	AMX, TET, AUG, NAL	1	2.5
		AMX, GEN, NAL, NIT, COT	1	2.5
		AMX, TET, AUG, NAL, NIT, COT	1	2.5
<i>Proteus rettgeri</i>	2	AMX, AUG, GEN, NIT, COT	1	2.5
		AMX, TET, AUG, GEN, NIT, COT	1	2.5
<i>Pseudomonas aeruginosa</i>	9	AMX, AUG, COT	2	5.0
		AMX, AUG, GEN	1	2.5
		AMX, AUG, NIT, COT	2	5.0
		AMX, AUG, GEN, NIT, COT	4	10.0
<i>Pseudomonas alkaligenes</i>	1	AMX, TET, AUG, GEN, NAL,	1	2.5
<i>Shigella dysenteriae</i>	1	AMX, TET, AUG, GEN, NAL, NIT, COT	1	2.5
<i>Shigella paradysenteriae</i>	1	AMX, AUG, GEN, NIT	1	2.5
<i>Shigella sonnei</i>	2	AMX, AUG, NAL, NIT	1	2.5
		AMX, TET, GEN, NAL, NIT	1	2.5

4. CONCLUSION

The study provided information on the water quality of selected swimming pools in Osogbo metropolis, Osun State, Nigeria. The microbiological quality of all the water sources were poor, and contaminated with different microbial organisms, they are pathogenic. High number of pool users call for more strict surveillance measures from responsible health authorities for protection of swimmers health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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