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ABSTRACT

BACKGROUND: Water recreational facilities like swimming pools attract people of all ages. However, these facilities are very suitable for the transmission of various microbial diseases and have been shown to pose public health concerns.

AIMS: This study assesses the presence of different Gram-negative bacteria pathogens and their antimicrobial susceptibility pattern in both private and public pools in Al-Ahsa.

METHODS: 11 private and 3 public recreational water facilities were sampled for the study. Collected water samples were inoculated into nutrient broth and incubated aerobically for 24 hours. The overnight growth was plated out on blood and MacConkey agars. Pure cultures of the bacteria samples were used for identification and antimicrobial susceptibility test using the Vitek 2 compactautomated system (BioMerieux, Marcy L'Etoile, France). Minimum inhibitory concentration was also provided by the Vitek 2 compact automated system.

RESULTS: 13 different Gram-negative bacteria species isolates were encountered in both pool types sampled. More of potential pathogens were isolated from the private than the public pools, of which *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* constituted 50% and 43%, respectively, of all the isolates. Findings also revealed a varied minimum inhibitory concentrations (MICs) indicating that the isolates were of different strains. Antibiotic susceptibility pattern also showed variability among the isolates.

CONCLUSIONS: This study has revealed a potential health risk associated with the use of water recreational facilities. The presence of *K pneumoniae* and *P aeruginosa* suggests a public health concern and should be looked into.

KEYWORDS: Public, private swimming pools, microbial, contamination

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Introduction

Swimming either in public or private pools is a sport generally enjoyed by all age groups particularly during warm seasons of the year. However, the sharing of swimming pools has been reported to lead to disease outbreaks.[1] This is despite the fact that there are guidelines that had been set by the World Health Organization (WHO) with the purpose of minimizing the risk of illnesses and infections originating from them. There have been reports of the contamination of swimming facilities by bacteria and other microbes, some of which have led to diseases of the eye, ear, skin, and digestive system.[2-4] Other reported diseases are infections associated with upper respiratory tract in immunocompromised patients[5] with the WHO[1] attributing such outbreaks to poor techniques at disinfection and sometimes the complete lack of disinfecting of these pools. Also, a report by Centers for Disease Control and Prevention (CDC) had pointed to an increase in the number of recreational water illnesses (RWIs) outbreaks for over a period of 2 decades.[6] This report attributed to the fact that chlorine treatment of swimming pools did not result in the killing of germs. It is stipulated that Protozoa parasites such as *Cryptosporidium* are capable of surviving for days in well-maintained pools with reported cases of diarrhea outbreaks increasing from 3411 cases in the year 2004 to 10 500 in the year 2008.[7] In addition to these, bacteria of *Pseudomonas* species are reported to be well adapted to surviving in a wide range of recreational water facilities despite having been disinfected. In a recent assessment of bacteria isolated from community showers before and after chlorination, it was reported that pseudomonal species were more chlorine-resistant than other bacteria species.[8] This report is contrary to the earlier views of Papadopoulou et al,[9] who stipulated that swimming pool–related disease outbreaks could be reduced if these pools are well managed. There are, however, other reports of antimicrobial resistance by bacteria isolated from swimming

pools against antiseptics and disinfectants, thus pointing to a public health problem that needs to be monitored carefully.[10]

Wide ranges of bacteria have been associated with both chlorine-treated and chlorine-free public and private swimming pools. Earlier research[11] found that bacteria from chlorinated water were more resistant to disinfectants than were those from un-chlorinated waters. Subsequently, many other research investigations have demonstrated chlorine-resistant bacteria in consumable water and water sources in general.[12,13] All these reports highlight the potential health problems posed by waterborne bacteria that are difficult to eradicate through conventional means of disinfecting and particularly so as drug-resistant genes are easily transferrable. Among the list of such bacteria are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* species, among others.[9] Also, some of these encountered bacteria isolates such as *Klebsiella pneumonia* and *P aeruginosa* exhibited multidrug resistance (MDR) against tested antibiotics.[14] Reasons given for such MDR by the researchers was that either the guidelines set by WHO aimed at minimizing the spread of infections in swimming pools were not being kept or that waterborne bacteria associated with recreational swimming pools had devised strategies to withstand eradication through disinfecting. Thus, the threat posed by MDR bacteria carrying drug-resistant genes cannot therefore be overlooked as they can be transferred, creating more public health problems.

Generally, the importance of hygiene in the prevention of bacterial infections cannot be overemphasized. In clinical settings, there are reports of pathogens which have shown decreased susceptibility to antiseptics.[15-17] Although such bacterial resistance against disinfectants in clinical settings are a major cause for nosocomial infections, their transference to either private of public swimming pools will be cause for public health concern.[18] Also, as diseases resulting from infections caused by *S aureus, E coli, Clostridium perfringens*, and *Enterococcus faecalis*, which had been isolated from different depths of pools by Saberianpour et al,[5] there is the possibility of transference of clinical-resistant genes to bacteria in communities through swimming pools. Humans continue to make water contact with bacteria-contaminated swimming pools even in an era when there is a global pandemic resulting from MDR bacteria pathogen. This makes it necessary for a more frequent and continuous monitoring of bacteria isolates from all types of swimming pools. The present investigation looks into characterization and antimicrobial susceptibility of Gram-negative bacteria isolates associated with some public and private swimming facilities in the region of the study.

Materials and Methods

Description of the study area

The study was carried in Al-Ahsa, named after the largest oasis in the world and is located between Riyadh and Dammam in the Eastern region of Saudi Arabia. Al-Ahsa has more than 10 000 hectares of arable land, for which concrete canals extending to more than 500 km were constructed. The water outlets into these canals are sometimes opened for the general populace for recreational swimming purpose at various times and seasons of the year. Water samples for the study were collected from these canals as well as from private swimming pools located in farm houses.

A total of 42 samples were collected from 3 public and 11 private pools located in Al-Ahsa region, all of which were given codes.

Ethical consideration

This study was approved by Deanship of Scientific research, King Faisal University (approval number 175081).

Collection of water samples

Water samples were collected from canals and private swimming pools in farmhouses of those who volunteered to have samples collected. Samples were collected from the surface and at depths of 30 cm, 50 cm from the surface at strategic positions of 30 cm from the edge of the pool as previously described,[4,20] as well as from the outlet where water was pumped out into the canal before any contact was made by members of the community. Sterile bottles were used for the collection, after which they were immediately transported to the microbiology laboratory in insulated ice package coolers.

Bacteria culturing

The standard methods for water sample examination were used for the analysis of collected water samples,[21] whereas bacteria plate count (BPC) was used for quality assurance.[22] Using a sterile swap, each water sample was inoculated into labeled bottles of nutrient broth and incubated aerobically at 37.5°C for 24 hours. The overnight growth were then plated out on both blood and MacConkey agars, incubated aerobically at 37.5°C for 24 hours. The spread agar plate technique was used for counting of colonies while pure samples of the microbial cultures of all phenotypically identified colonies[23,24] were prepared and used for the identification of the Gram-negative bacteria isolates using the Vitek 2 Compact automated system (BioMerieux, Marcy-l'Etoile, France) according to manufacturer's guidelines.

Bacteria identification and antimicrobial susceptibility test

Isolates were identified using the Vitek 2 Compact automated system (BioMerieux). A sterile applicator stick was used to transfer a sufficient number of colonies of pure culture of the micro-organism and suspended in a 3-mL sterile saline test tube. The appropriate turbidity was determined based on the manufacturers' guidelines (0.50-0.63) using the turbidity meter, DensiChek™ (BioMérieux Inc DensiCHECK™), according

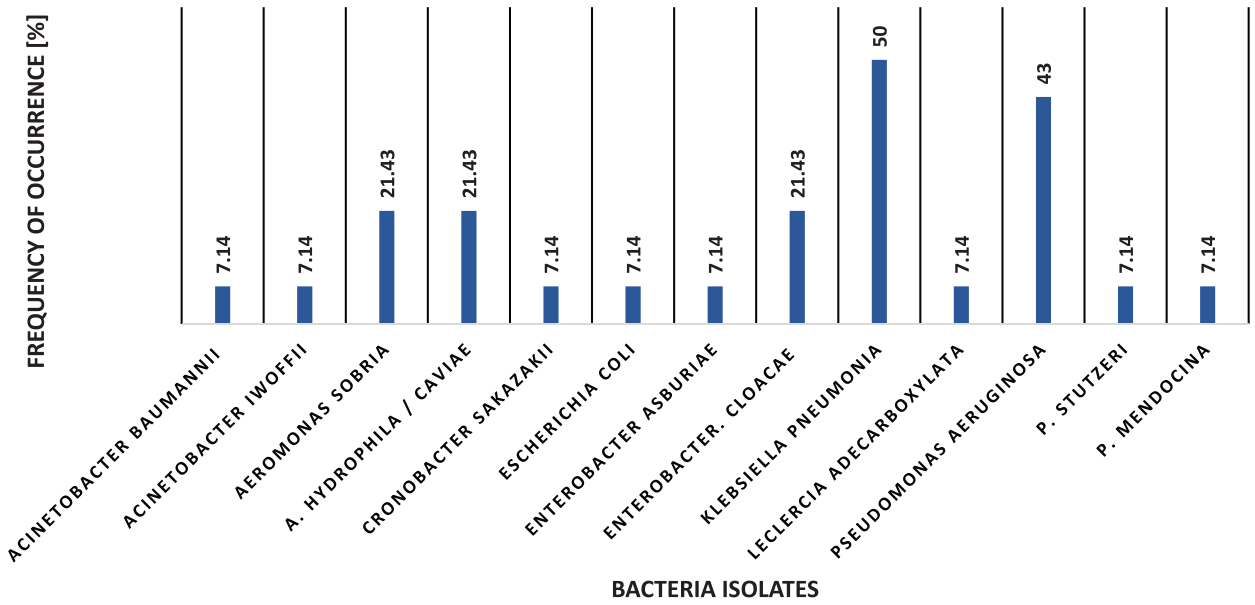

Figure 1. Frequency distribution of encountered bacteria isolates from public and private water recreation pools.

to the manufacturer's guidelines. Identification cards were then inoculated with the suspension of the microorganism and placed the cassette with the identification card in the neighboring slot. The Gram-negative cards were used for the identification of Gram-negative isolates. The minimum inhibitory concentrations (MICs) and resistance pattern were determined with the Vitek 2 Compact automated system using the AST-N307 cards. The following antibiotic disks were used for the study: ampicillin (10 µg), amikacin (30 µg), gentamicin (10 µg), amoxicillin/clavulanic acid (20/10 µg), cefalexin (30 µg), cefazolin (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), nitrofurantoin (300 µg), tigecycline (15 µg), and trimethoprim/sulfamethoxazole (1.25/ 23.75 µg). Results were interpreted using the M100-S25 of Clinical and Laboratory Standards Institute (CLSI) (2015). The Minimum inhibitory concentrations (MICs) and antimicrobial susceptibility patterns were determined using the Vitek 2 Compact automated system.

Statistical analysis. Data were analyzed using excel graphics with results expressed as percentage frequencies. Comparison of isolate sources and antibiotics susceptibility are expressed in terms of percentage.

Results

The following Gram-negative bacteria isolates were encountered in the study: *Pseudomonas aeruginosa, Pseudomonas stutzeri, Pseudomonas mendocina, Klebsiella pneumoniae, Acinetobacter baumannii* complex, *Acinetobacter lwoffii, E coli, Aeromonas sobria, Aeromonas hydrophila/caviae, Leclercia adecarboxylata, Enterobacter asburiae, Enterobacter cloacae* complex, and *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*). The percentage frequency of occurrence of these isolates is shown in Figure 1. The figure shows *K pneumoniae* to be the most encountered bacteria constituting of 50% of the isolates. The cumulative frequency of occurrence of all pseudomonal species was 57.14%, of which *P aeruginosa* made up 43% of the isolates. A comparison of the types of bacteria isolates from the private and public swimming facilities is shown in Table 1. There were 13 Gram-negative bacteria isolates encountered, and they are shown in Table 1. Ten (77%) of these were isolates from private pools, whereas 5 (39%) isolates were isolated from the public pools. All *K pneumoniae* isolates were from private pools, whereas *P aeruginosa* was encountered from both public and privately used pool. Also, *Acinetobacter* species encountered in the study were isolated from private pools as shown in Table 1.

An overall antimicrobial resistance against tested antibiotic was as follows: cefazolin 81%, cefalexin 76.2%, ampicillin 67%, cefuroxime axetil 57%, cefuroxime 57%, amoxicillin/clavulanic acid 38%, ampicillin/sulbactam 33%, and nitrofurantoin 24%. There was a 14.3% resistance exhibited against tigecycline and meropenem each by the isolate, whereas 4.46% of were found to be resistant to imipenem and the results are shown Figure 2. The results on the minimum inhibitory concentration values to the antibiotics are shown in Table 2, whereas results of susceptibility of the isolates against the tested antibiotics are shown in Figure 3. Of the listed 16 antimicrobials used in the investigation, *P aeruginosa* were resistant to 13 (81%), *Acinetobacter* species were resistant to 12 (75%), whereas *K pneumoniae* exhibited resistance to 9 (56%) of the antimicrobials. The figure also shows that resistance to the carbapenems and tigecycline was seen only among *Pseudomonas* and *Acinetobacter* species. Also, depending on the depth of pool of bacteria collection, there was no specific pattern of microbial susceptibility against the tested antibiotics as is shown in the results presented in Table 3.

Discussion

The public health risk for the transmission of bacterial infections through swimming pools is highlighted by the results of this study. This problem is more emphasized with private

Table 1. Comparison of bacteria isolates between private and public recreational water facilities.

BACTERIA ISOLATE	TYPE OF SWIMMING POOL	
	PRIVATE, N = 11 (%)	PUBLIC, N = 3 (%)
Acinetobacter baumannii complex	1 (9.1)	0 (0)
Acinetobacter lwoffii	1 (9.1)	0 (0)
Aeromonas sobria	0 (0)	3 (100)
Aeromonas hydrophila/caviae	3 (27.3)	0 (0)
Cronobacter sakazakii	1 (9.1)	0 (0)
Escherichia coli	1 (9.1)	0 (0)
Enterobacter asburiae	0 (0)	1 (33.33)
Enterobacter cloacae complex	2 (18.2)	1 (33.33)
Klebsiella pneumoniae	7 (63.63)	0 (0)
Leclercia adecarboxylata	1 (9.1)	0 (0)
Pseudomonas aeruginosa	4 (36.4)	2 (66.67)
Pseudomonas stutzeri	1 (9.1)	0 (0)
Pseudomonas mendocina	0 (0)	1 (33.33)
Total number of bacteria species	10/13 (77%)	5/13 (39%)

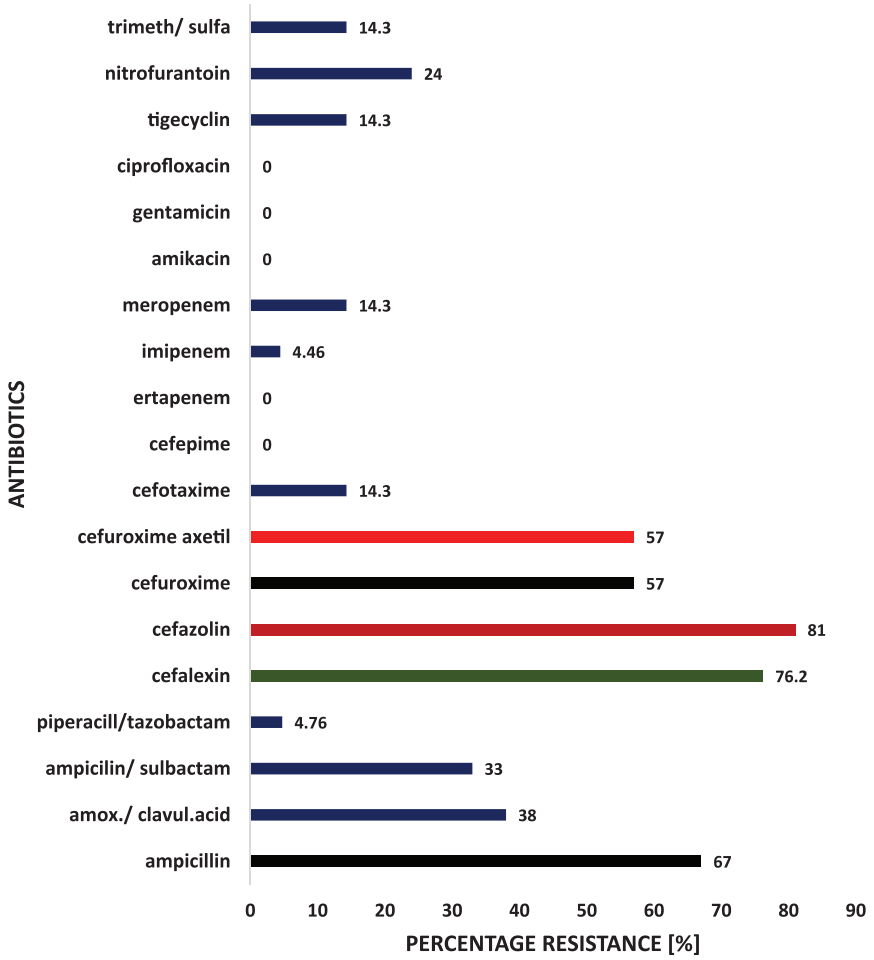

Figure 2. Showing percentage antimicrobial resistance against the tested antibiotics.

Table 2. Minimum inhibitory concentration for selected antibiotics used in treatment of Gram-negative bacteria.

BACTERIA ISOLATE	MINIMUM INHIBITORY CONCENTRATIONS FOR SELECTED ANTIBIOTICS											
	AUG	AMS	PTZ	CZ	CXM	CFT	CFL	IMP	MER	AMK	GEN	CIP
Pseudomonas aeruginosa [1]	⩾32	⩾32	16	⩽4	⩾64	2	⩾64	2	⩾16	⩽2	⩽1	⩽0.25
P aeruginosa [2]	4	⩾32	⩾128	⩽4	⩾64	2	⩾64	⩾16	⩾16	⩽2	⩽1	⩽0.25
P aeruginosa [3]	8	⩾32	8	⩾64	⩾64	⩾64	8	2	0.5	⩽2	⩽1	⩽0.25
P aeruginosa [4]	4	4	⩽4	⩾64	ND	8	⩾64	ND	⩽0.25	⩽2	⩽1	⩽0.25
P aeruginosa [5]	⩾32	⩾32	8	⩽4	⩾64	⩾64	4	2	⩽0.25	⩽2	⩽1	⩽0.25
P aeruginosa [6]	32	32	32	8	⩽64	⩽64	2	2	0.5	2	1	0.25
Aeromonas sobria	4	⩾32	⩽4	8	⩽1	⩽1	⩾64	8	⩾16	⩽2	⩽1	⩽0.25
A sobria	4	⩾32	8	⩾64	⩽1	⩽1	⩽16	8	8	⩽2	⩽1	⩽0.25
A sobria	⩽2	16	⩽4	⩽4	⩽1	ND	⩾64	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
Aeromonas hydrophila/caviae	4	⩾32	⩽4	⩾64	⩽1	⩽1	⩾64	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
A hydrophila/caviae	4	16	⩽4	⩾64	2	⩽1	⩽1	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
Cronobacter sakazakii	⩽2	⩾32	⩽4	⩽4	8	⩽1	8	⩽.25	⩽0.25	⩽2	⩽1	⩽0.25
Enterobacter cloacae	⩾32	ND	8	⩾64	4	⩽1	⩾64	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
E cloacae	⩾32	ND	⩽4	⩾64	16	⩽1	⩾64	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
E cloacae	⩾32	ND	⩽4	⩾64	16	⩽1	⩾64	1	⩽0.25	⩽2	⩽1	⩽0.25
Klebsiella pneumoniae [1]	⩽2	⩽2	⩽4	4	8	⩽1	⩾64	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
K pneumoniae [2]	⩽2	4	⩽4	⩽4	4	⩽1	⩽4	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
K pneumoniae [3]	4	16	⩽4	⩽4	8	⩽1	8	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
K pneumoniae [4]	16	⩽2	⩽4	⩽4	4	⩽1	⩽4	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25
E coli	⩽2	4	⩽4	⩽4	4	⩽1	8	⩽0.25	⩽0.25	⩽2	⩽1	⩽0.25

Abbreviations: Amk, amikacin; Ams, ampicillin/sulbactam; Aug, amoxicillin/clavulanic acid; Cft, cefotaxime; Cip, ciprofloxacin; CXM, cefuroxime; CZ, cefazolin; Gm, gentamicin; Imp, imipenem; Mer, meropenem; ND, not done; Ptz, piperacillin/tazobactam.

swimming pools in the region of the present investigation than those shared public recreation pools. Similar findings had been reported by researchers[19,24] and in different regions of the world.[9] The findings on the isolation of *K pneumoniae, P aeruginosa, Acinetobacter* species, *E coli*, among other bacteria species are similar to those of Mansoorian et al,[25] who reported that the most important bacteria isolates in their research were *P aeruginosa, K pneumoniae, Pseudomonas species, E coli*, and species of *Acinetobacter*. However, although they had encountered these bacteria on the surfaces of both private and public pools, the findings in the present report showed *P aeruginosa* and other pseudomonal species to be the most commonly encountered species in both the public and private pools. Differences could be attributed to regional methods used for the disinfecting of the pools as well as possible difference in the response circulation bacteria clones the disinfecting agents.

Also worth noting, however, is that there were more types of bacteria species isolated from the private swimming pools in the present investigation than were those isolated from the public ones.

That all the *K pneumoniae* in this study were from private pools is a public health risk as these pools are usually visited by holiday makers who rent the farm houses where the pools are located. This is consistent with the findings of Papadopoulou et al,[9] who reported the isolation of *K pneumoniae* from in-door swimming pool, from where they had also encountered the highest percentage of multi-antibiotic-resistant bacteria. Although healthy people do not usually get *K pneumoniae* infections, the bacterium could, however, constitute a health problem to users particularly when it gains entry into the respiratory system or in the case of users who are immunocompromised, where the bacterium could cause pneumonia.

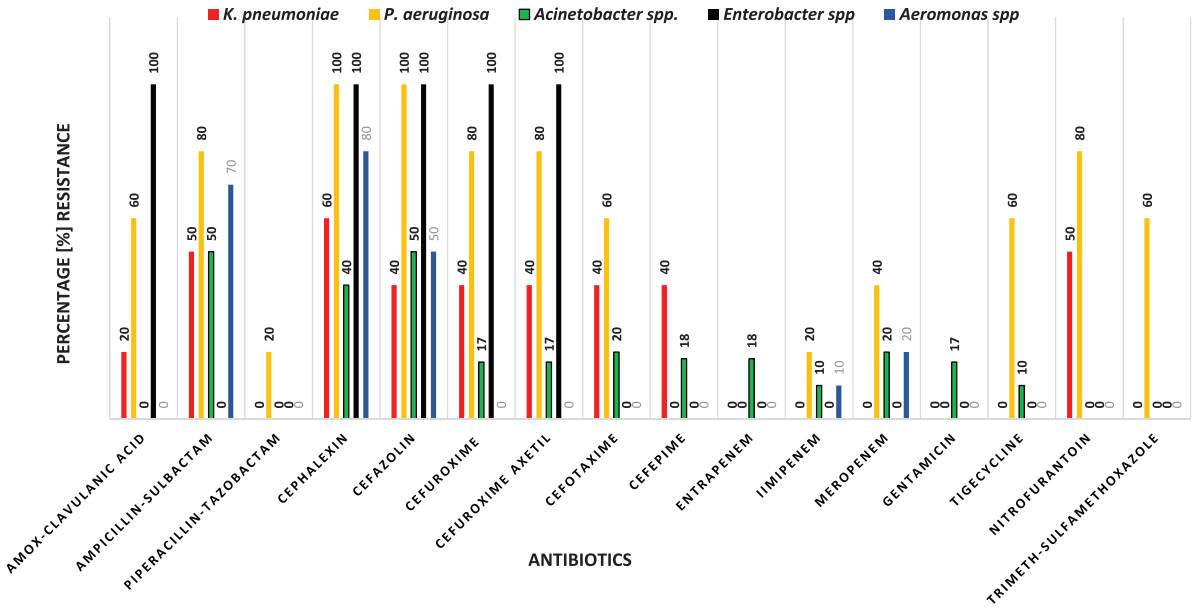

Figure 3. Comparison of percentage resistance by Gram-negative isolates against antibiotics of choice in treatment.

Generally, therefore, the high frequency of bacteria isolation from private pools suggests the probability of non-compliance to WHO recommendations for the disinfection swimming pools. Thus, the differences in disinfecting techniques between owners of private pools and those of the public pools could explain why there were such variations in the number of isolated bacteria species as seen from the results in the region of the present investigation. However, that *P aeruginosa* and other species of *Pseudomonas* were isolated from both the private and public swimming pools in this investigation, could be attributed to the ubiquitous nature of the bacterium at being able to survive in moist environments as well as resist antiseptics and antimicrobial.[26] Reports, on pool contamination by pseudomonal species, that vary as similar to the present findings are those of other researchers in other regions of the world.[5,14] However, contrary to the results of the present report are those of other researchers.[19] Tirodimos et al[14] found the prevalence of *P aeruginosa* to be low in their investigation as well as their isolates displaying a low antibiotic resistant pattern. They stipulated that the low antimicrobial resistance by *P aeruginosa* isolates in their study was indicative of area of low antibiotic usage.[14] The 43% frequency of occurrence for *P aeruginosa* shown in the results of this investigation is similar to that of 42% earlier reported.[19] Differences in findings could be attributed to regional variation as well as a number of other possible contributory factors. There is the possibility of non-compliance with the chlorination standards set by the WHO in some cases as the private pools in this report were more contaminated than those of the public ones. The maintenance of the public pools appears to be better than what is likely the case of those own privately. Alternatively, there could be the probability of resistance to chlorine as had been reported by researchers from other regions of the world.[14] However, literature is silent on such findings in the region of this study.

In terms of the susceptibility to antimicrobial drugs, the 81% resistance seen with *P aeruginosa* isolates is high. In our earlier report on *P aeruginosa* clinical isolate from the region of the present investigation, a growing resistance to anti-pseudomonal drugs was observed and reported with the caution to a close monitoring of this bacterium in the this south-eastern region of Saudi Arabia.[27] Tirodimos et al[14] had also found the *P aeruginosa* isolates in their research to be highly resistant to antimicrobials and attributed this to be due to the fact that the bacterium is naturally resistant to many antibiotics including those of first and second cephalosporins, first-generation fluoroquinolones among a wide range of other antimicrobials. However, that these bacteria isolates are environmentally isolated samples could constitute of a public health concern that needs monitoring.

There is a view that high antimicrobial resistance by environmental bacteria isolates could be as a result of uncontrolled disposal of antibiotics into the environment or that the disposal of hospital waste has created environmental bacteria carrying clones exhibiting high antimicrobial resistance.[24] The report indicated the development of adaptive process engaged by bacteria which alter colony traits that help increase their virulence and resistance to antimicrobials in the environment. There is the probability that the encountered pseudomonal species are carrying antimicrobial-resistant clones of clinical origin. This more so as the region of this study is known generally for bacteria strains that are highly resistant to antibiotics. It is also worth noting that resistance to the carbapenems and tigecycline which was associated with *A baumannii*, other *Acinetobacter* species, and *P aeruginosa* could be pointing to the carrying of resistant clinical clones by these environmental isolates. *Acinetobacter*, an opportunistic pathogen affecting immunocompromised patients, has received much attention by researchers in the

Table 3. Comparison of antimicrobial susceptibility of bacteria isolates in relation to the source of isolation.

BACTERIA ISOLATE	ISOLATE ID	SOURCE OF ISOLATION/DEPTH	PERCENTAGE (%) RESISTANCE	PERCENTAGE (%) INTER.	PERCENTAGE (%) SENSITIVE
Klebsiella pneumoniae	Kp1	Pr1d	5.3	5.3	89.4
	Kp2	Pr2s	5.3	0.0	94.7
	Kp3	Pr3d	31.6	15.8	52.6
	Kp4	Pr4d	6.0	0.0	94
	Kp5	Pr5d	0.0	12.0	88
	Kp6	Pr6s	11	0	89
	Kp7	Pr7s	5.3	0	94.7
	Kp8	Pr8d	26.32	15.79	57.89
	Kp9	Pr9d	0.0	0.0	100
	Kp10	Pr10d	5.3	11	83.7
	Kp11	Pr11	6.0	0.0	94
Pseudomonas aeruginosa	Ps1	Pb3m	67	11	22
	Ps2	Pb7m	61.1	6.0	33.33
	Ps3	Pb7d	39	0.0	61
	Ps4	Pr1d	33	0.0	67
	Ps5	Pr2s	61	6.0	33
	Ps6	Pr3s	50	10	40
Aeromonas sobria	As1	Pb1.1	5.26	5.26	89.48
	As1	Pb7.1	17	11	72
	As3	Pb7.2	28	5.56	66.44
Aeromonas hydrophila/caviae	Ahc1	Pr8d	16.7	5.55	7.8
	Ahc2	Pr8s	22	0	88
	Ahc3	Pr8d	22	5.56	72.44
Enterobacter species	Ent 1	Pbs1	23.5	11.76	64.74
	Ent 2	Pb7m3	29.41	0.0	70.54
	Ent 3	Pb7m3	29.41	6.0	64.59
	Ent 4	Pr7s	29.42	17.64	52.94

Abbreviations: d, 50 cm depth; m, 30 cm depth; pb, public pool; pr, private pool; s, surface.

region of the present investigation in recent years.[28] The study, therefore, shows the potential health risk associated with use of these water recreational facilities and that *K pneumoniae* and *P aeruginosa* are the most frequently encountered isolates suggests that there is a need for monitoring.

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Author Contributions

Research concept was by

FHT: Field collection of samples, Laboratory experiments

AAM: Research concept, Field collection of samples, Laboratory experiments

LBE: Laboratory experiments, Data Analysis of results and article write-up

PME. Research concept, Data Analysis of results and article write-up

MMI: Bacteriological experiments, final preparation of report

All researchers reviewed the write up and searched for literature as well as updating literature when there was the need to.

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