Isolation of Antibiotic-Resistant Bacteria From The Atmospheric Air In Hospital Wards And Outdoor Areas In Kuwait During Sandstorms

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Abstract

Aim: Antibiotic resistance is a public health concern that is linked to increased mortality, morbidity, extended hospital stays, decreased productivity, and therefore, increased financial implications. The source and dissemination of antimicrobial resistance have been linked to environmental factors, including dust storms. There is clear evidence that there has been a rapid increase in temperature in the past decades which increases the risk of sandstorms in places that had never previously experienced this phenomenon. The aim of this study is to isolate medically important micro-organisms in atmospheric air samples from outdoor spaces and inside hospital wards during sandstorms and to characterize their antimicrobial sensitivity profiles to common antibiotics.

Findings: Eighty-four colonies were isolated from the target sites and identified as *Staphylococcus aureus*, coagulase-negative *Staphylococcus*, *Bacillus* spp., *Acinetobacter* spp., from hospital air samples, and *Pseudomonas* spp., and *Staphylococcus* spp. from outdoor air samples. Multi-drug resistance patterns were observed for isolates obtained from both indoor and outdoor air samples. PCR showed 6.5% of *S. aureus* contained *mecA*.

Conclusion: This finding supports the notion that atmospheric air during dust storms can be counted as a source of antibiotic resistant bacteria which merits more attention especially with global warming and climate change contributing to extreme weather events.

Introduction

In the current clinical settings, antibiotic resistance and its effects are the greatest healthcare concerns. The latest body of evidence implicates the environment as a critical element in transmitting and evolving antibiotic-resistant bacteria [1]. However, clear evidence that directly links the evolutionary and ecological factors to the emergence of resistant genes in microbes is currently lacking. Therefore, such research gaps call for more lucid explanations of the development and evolution of resistant genes, their mobilisation, transfer, and dissemination in the environment.

Antimicrobial resistance is responsible for a large proportion of mortalities on an annual basis. Research has suggested a projection in its increase in the coming years, which has led the World Health Organization (WHO) to make a recognition regarding its threats as a public health hazard [2]. As far as the history of recognising danger and endeavours against antimicrobial resistance is concerned, activities aimed at controlling the development of resistance have primarily been initiated in the clinical settings and at the community level. Very recently, the problem has also commenced in the environmental and agricultural context [3]. The aim to solve this problem is the reduction of the transmission and prevention in selecting the resistant bacteria, whilst undertaking antibiotic therapies.

WHO has issued the warning that the modern world is fast heading to the post-antibiotic times, leading to uncontrollable morbidity and mortality [4]. Furthermore, the hospitals and healthcare facilities are fast turning into the hubs of extremely drug-resistant microorganisms [5], whereby even routine surgical procedures like cancer surgery and other surgical operations may become extremely risky. A scientific report forecasts that by the middle of the twenty-first century, under the current circumstances, if there is no improvement in the antibiotic-resistance development scenario, the population figures of the world shall be from 11 million to 440 million lesser than projected. Similarly, the world's economic losses will make the economy shrink by anywhere ranging from 0.06–3.1% [6]. Antimicrobial resistance is normally linked to substantial mortality, morbidity, extended hospital stays, and higher financial implications.

Climate change and its effect on environment and microbiome are important issues of concern. The insufficiency of such a knowledge base regarding how, why, and when the environmental factors become contributory to the development of resistance make antimicrobial resistance risk reduction quite a difficult task. For example, unidentified microbes transported by desert dust storms across the Atlantic were shown to be the causative agents in coral diseases, contributing to the decline of reef ecosystems in the Caribbean basin [7]. The involvement of environmental factors as contributors to sourcing and dissemination of antimicrobial resistance in clinical settings has gained considerable recognition. The latest body of evidence implicates the environment as a critical element in transmitting and evolving antibiotic resistant bacteria [1]. For example, shunt infections in the operating theatres were caused by airborne bacteria rather than originating from patients' skin [8–9] and also contamination in operating theatres air conditioning systems was to blame for some post-cataract infections in a hospital [10].

Kuwait is a country in the western part of the Asian continent, with vast deserts and an abundance of dust storms in hot and dry weather conditions. A greater part of the country consists of deserts and an abundance of dust storms in hot and dry weather conditions. A research study based on the desert of Kuwait found 147 colony-forming units (CFU) in the desert dust [11]. Global warming has affected this region with higher temperatures (1.5^0C to 2^0C increase in temperature annually), and increase in the frequency of rising dust and longer duration of sandstorms (https://www.ecomena.org ›climate-change-kuwait).

It also faces risks from sea level rise, lack of rainfall, and biodiversity degradation (https://thearabweekly.com/climate-change-endangers-quality-life-kuwait).

Our aim in this study was to identify if any antibiotic resistant bacteria that are significant in clinical settings were found in air during sandstorms.

Materials And Methods

Sampling

Air sampling was performed in two hospitals (X and Y) in Kuwait Air samples were collected from common areas of different departments; the Intensive Care Unit (ICU), Cardiac Care Unit (CCU), and Operating Theatres (OT) with consideration of beds, patients, and staff numbers in each department. The temperatures ranged between 18^0C–20^0C. The ICU and CICU (cardiac ICU) had bed capacity of 40 and 4 respectively. Only one operating theatre was available for sample collection. Thirty-four airborne isolates of dust raised during sandstorms were collected from open areas of two different sites (Site 1 and Site 2).

Sample collection was achieved by SKC Biostage™ device onto R2A agar (Reasoner's 2A agar - a culture medium for *the bacterial examination of drinking water)* and nutrient agar (culture medium used for the growth of non-fastidious bacteria) containing 50µg/ml cycloheximide, 10µg/ml nystatin and 10µg/ml of one of these antibiotics: erythromycin, streptomycin, tetracycline and nalidixic acid.

Samples were collected in triplicates from each target site; thirty-four collected from an open air site during a sandstorm and thirty-nine from the two target hospitals from inpatient departments; operating theatres, intensive care units, and cardiac care units of the two hospitals equipped with high-efficiency particulate air (HEPA) filtration systems.

Single colonies from the collected samples were isolated and incubated for 3-4 days at 37° C to enhance growth. Upon visible growth, each isolate was put in an antibiotic plate and stored with glycerol supplement at -80° C (Table 1).

Identification Of Airborne Bacteria

Genomic DNA from single isolated colonies from each antibiotic plate were extracted using Wizard genomic DNA purification kit (promega). Amplifications of 16S rRNA were carried out in 50µl reaction with 1-2µl lyzed cell sample, 5X FIREPol Master Mix, 10µM each primers 27F (AGAGTTTGATCCTGGCTCAG), and 1492R (GGTTACCTTGTTACGACTT) under the following conditions: 5 min at 95°C followed by 35 cycles of 30s at 94°C, 30s at 54°C, and 2 min at 72°C, and 1 cycle of 10 min at 72°C. The PCR products were separated on 1% agarose gel. Amplified DNA fragments were purified using Wizard SV Gel & PCR purification kit (Promega). The PCR amplicons were then sequenced using ABI 3130xl genetic analyser. BioNumerics v.7.1 (Applied Maths, Ghent, Belgium) was used to analyse the sequences. Identification at the species level was performed by comparison with the Ribosomal Database Project database (http://rdp.cme.msu.edu/) and by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antibiotic-resistance Screening

The susceptibility of the bacterial isolates to different antibiotics was determined in triplicate using the standard disk diffusion and E-test methods and analysed (CLSI[24] and EUCAST).

Detection of mecA **gene**

PCR of *mecA* gene was carried out in 50µl reaction with 1-2µl lyzed cell sample, 5X FIREPol Master Mix, 10µM each primer forward 5'-AGGCCCGGGAACGTATTCAC-3' and reverse 5'-GAGGAAGGTGGGGATGACGT-3' under the following conditions: an initial denaturation step at 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec, with a final extension at 72°C for 5 min. The PCR products were separated on 1% agarose gel. Amplified DNA fragments were purified using Wizard SV Gel & PCR purification kit (Promega). The sequences were analysed using BLAST (http://blast.ncbi.nlm.nih.gov)

Results

A total number of 84 colonies were isolated from 34 air sample agar plates. These isolates are shown is Table 1.

Table 1
The number of bacteria identified in samples.

Sample	Number
Staphylococci	46
Bacillus	20
Acinetobacter	5
Pseudomonas	2
Others	11
Total	84

Hospital Air Samples

Staphylococci **(n=26)**

Twenty six*Staphylococci* were recovered from hospital air samples Including 7 *S. epidermidis*, 6 *S. capitis*, 5 *S. hominis*, 2 *S. haemolyticus* and 2 *S. warneri*, 1 *S. caprae*, 1*S. petrasii*, 1*S. aureus*, and 1 *S. lugdunensis*.

The graph in (Figure.1) shows the overall resistance patterns of *Staphylococcus* species obtained and cultured from air against medically important antibiotics used in clinical settings. Majority (73%, 19 out of 26) of the *Staphylococcus* colonies were resistant to trimethoprim-sulfamethoxazole followed by 50% resistant to erythromycin, and 23% resistant to ciprofloxacin. 19% of the *Staphylococci* isolates were resistant to cefoxitin, whilst 15% were resistant to tetracycline and clindamycin. A smaller number were resistant to oxacillin (12%), kanamycin (12%), linezolid (8%), streptomycin and vancomycin (4% each).

Notably, all *S. epidermidis* isolates were found to be resistant to trimethoprim-, sulfamethoxazole whilst most (4 out of 7 isolates) were resistant to at least three different classes of antibiotics, including trimethoprim-sulfamethoxazole, erythromycin, tetracycline, kanamycin, clindamycin, and ciprofloxacin. Nevertheless, only one isolate showed resistance to oxacillin.

S. capitis isolates were resistant to trimethoprim-sulfamethoxazole and erythromycin but sensitive to oxacillin.

57% (n=5) of the *S. hominis* isolates also showed resistance to trimethoprim-sulfamethoxazole. Two of these were found to show multiple drug resistance pattern, including oxacillin.

The two *S. haemolyticus* isolates showed cross-resistance to trimethoprim-sulfamethoxazole and erythromycin and were also found to be resistant to another class of antibiotic (either ciprofloxacin or tetracycline), but not to oxacillin. The rest of the *Staphylococci* isolates showed resistance to only one or two antibiotic classes (Table 2).

Table 2
Resistance patterns of *Staphylococcus* numbers isolated from hospital air samples.

Antibiotic	Numbers
Amikacin (AK)	0
Erythromycin (EM)	13
Cefoxitin (FX)	5
Gentamicin256 (GM256)	0
Gentamicin1024 (GM1024)	0
Linezolid (LZ)	2
Oxacillin (OX)	3
Trimethoprim-Sulfamethoxazole (SXT)	0
Streptomycin (STP/SM)	1
Tetracycline (TET/TC)	4
Trimethoprim (TR)	19
Vancomycin (VA)	1
Kanamycin (KM)	3
Clindamycin (CM)	4
Ciprofloxacin (CI)	6

Bacillus **(n=8)**

Eight isolates of *Bacillus* were obtained and cultured from hospital air samples (Table3), consisting of 5 *B. subtilis*,1 *B. cereus*, 1 *B. clausii*, and 1 *B. haynassu*.

63% (n=)of the *Bacillus* isolates were found to be resistant to oxacillin, 38% (n=)were resistant to erythromycin, and another 38% (n=)were resistant to trimethoprim-sulfamethoxazole. 25% (n=)were resistant to mupirocin, whilst the following antibiotics were found to have one (13%) isolate to which it was resistant to:cefoxitin, streptomycin, tetracycline, vancomycin, kanamycin, and clindamycin (Figure.2). Analysis for the resistance pattern (based on MIC per species indicates three out of five isolates of *B. subtilis* showed resistance to oxacillin. Two of these isolates showed multiple drug resistance patterns. *B. cereus* isolate was resistant to oxacillin and trimethoprim, whilst *B. haynassu* was resistant to mupirocin and streptomycin. *B. clausii* isolate showed multiple drug resistance, including erythromycin, cefoxitin, oxacillin, vancomycin, kanamycin, and clindamycin (Table 3).

Table 3
Resistance pattern of *Bacillus* numbers isolated from hospital air samples.

Antibiotic	Numbers
Amikacin (AK)	0
Erythromycin (EM)	3
Cefoxitin (FX)	1
Gentamicin256 (GM256)	0
Gentamicin1024 (GM1024)	0
Mupirocin (MU)	2
Oxacillin (OX)	5
Streptomycin (STP/SM)	1
Tetracycline (TET/TC)	1
Trimethoprim (TR)	3
Vancomycin (VA)	1
Kanamycin (KM)	1
Clindamycin (CM)	1

Acinetobacter **(n=5)**

There were five isolates of *Acinetobacter* cultured from hospital air, four of which were *A. baumanii*, whilst the other one was *A. lwoffii* . 80%(n=) of *A. baumanii* were resistant to imipinem, meropenem, and tetracycline while 40% (n=1) were resistant to amikacin (Figure.3). This also implies multiple drug resistance patterns in *A. baumanii*. *A. lwoffii* did not show resistance to any of the tested antibiotics (Table 4).

Table 4
Resistance pattern of *Acinetobacter* numbers isolated from hospital air samples.

Antibiotic	Numbers
Amikacin (AK)	2
Imipenem (IP)	4
Meropenem (MP)	4
Tetracycline (TET/TC)	4

Other Isolates

There was a total of ten other isolates obtained from hospital air samples. These consisted of *Micrococcus aloeverae*, *Micrococcus luteus*, *Corynebacterium simulans*, *Luteimonas terrae*, *Agrobacterium salinitolerans*, *Corynebacterium amycolatum*, *Corynebacterium casei*, and *Escherichia fergusonii*. All of these isolates, except for *L. terrae*, *C. casei*, and *E. fergusonii*, showed resistance to Mupirocin. Moreover, multiple drug resistance was exhibited by *A. salinitolerans*, *M. aloeverae*, and *C. amycolatum*.

Outdoor Isolates

Bacillus **(N=12)**

Ten out of the twelve isolates across all the species showed resistance to at least three different classes of antibiotics, including Oxacillin. Based on MIC values, three showed resistance to imipenem, vancomycin, and linezolid and only one showed resistance to meropenem, amikacin, and ciprofloxacin, and none showed resistance to colistin and gentamicin (Table 5).

Both *Solibacillus isronensis B3W22* isolates showed resistance to erythromycin, cefoxitin, imipenem, mupirocin, oxacillin, streptomycin, tetracycline, and trimethoprim (Figure.4).

Table 5
Resistance pattern of *Bacillus* numbers isolated from outdoor air samples.

Antibiotic	Numbers
Amikacin (AK)	1
Ciprofloxacin (CI)	1
Colistin (CT)	0
Erythromycin (EM)	6
Cefoxitin (FX)	5
Gentamicin256 (GM256)	0
Imipenem (IP)	3
Linezolid (LZ)	3
Meropenem (MP)	1
Mupirocin (MU)	7
Oxacillin (OX)	10
Streptomycin (SM)	5
Ticarcillin (TC)	3
Teicoplanin (TP)	1
Trimethoprim (TR)	7
Vancomycin (VA)	3

Pseudomonas **(N=2)**

Two isolates of *Pseudomonas* spp. were obtained consisting of *P. xanthomarina* and *P. xiamenensis DSM22326*. Both showed resistance to Imipenem. *P. xiamenensis* exhibited multiple drug resistance pattern, including resistance to ciprofloxacin, linezolid and imipenem (Table 6) (Figure 5).

Table 6
Resistance pattern of *Pseudomonas* numbers isolated from outdoor air samples.

Antibiotic	Numbers
Amikacin (AK)	0
Ciprofloxacin (CI)	1
Erythromycin (EM)	0
Cefoxitin (FX)	0
Imipenem (IP)	2
Linezolid (LZ)	1

Staphylococcus **(N=20)**

A total of 20 isolates were obtained from outdoor air samples. These consisted of the following: three *S. arlettae*, three *S. epidermidis*, three *S. hominis* and three *S. xylosus*, two *S. cohnii* and three *S. haemolyticus*, and one *S. aureus*, 1 *S. equorum*, 1 *S. piscifermentans*, and 1 *S. saprophyticus*.

95% (19 out of 20 isolates) showed resistance to oxacillin, 65% (13 out of 20) to trimethoprim and 60% (12 out of 20) were resistant to erythromycin. 40% (8 out of 20 isolates) showed resistance to teicoplanin, and 35% (7 out of 20) showed resistance to cefoxitin and mupirocin. There was 20% (4 out of 20) resistance to clindamycin as well as imipenem, whilst there was 15% (3 out of 20) resistance to tobramycin. Additionally, there were 10% (2 out of 20) resistance to each of chloramphenicol, kanamycin, sulfamethoxazole-trimethoprim, and 5% (1 out of 20) resistance to each of amikacin and ciprofloxacin (Figure 6).

All the *S. arlettae* isolates exhibited cross-resistance to oxacillin and trimethoprim, and all three showed multidrug resistance patterns. However, none was resistant to vancomycin, imipenem, ciprofloxacin, and linezolid.

For *S. epidermidis*, *S. hominis*, and *S. xylosus* isolates, aside from all being resistant to oxacillin, only one isolate from each species showed resistance to less than three drug classes. All *S. haemolyticus* isolates also showed multiple drug resistance patterns. In fact, only 5 out of the 20 isolates showed resistance to only two classes of antibiotics. Nevertheless, none of these isolates exhibited resistance to gentamicin, linezolid, teicoplanin, and vancomycin (Table 7).

Table 7
Resistance pattern of *Staphylococcus* numbers isolated from outdoor air samples.

Antibiotic	Numbers
Amikacin (AK)	1
Ciprofloxacin (CI)	1
Chloramphenicol (CL)	2
Clindamycin (CM)	4
Erythromycin (EM)	12
Cefoxitin (FX)	7
Gentamicin256 (GM256)	0
Gentamicin1024 (GM1024)	0
Imipenem (IP)	4
Kanamycin (KM)	2
Linezolid (LZ)	0
Meropenem (MP)	7
Oxacillin (OX)	19
Trimethoprim-Sulfamethoxazole (SXT)	2
Tobramycin (TM)	3
Ticarcillin (TC)	8
Teicoplanin (TP)	0
Trimethoprim (TR)	*13*
Vancomycin (VA)	0

Detection and Amplification of mecA **Gene PCR**

All *S. aureus* isolates were screened for the presence of *mecA* gene by PCR. *mecA* gene was detected in only 3 *S. aureus* - (6.5%) isolated from ICU.

Discussion

Kuwait, a country in the Gulf area of the Middle East (southwest Asia), is mostly a dry desert with regular sandstorms. Occasionally, sandstorms could result in closures of operating theatres due to high levels of dust in the atmospheric air. Air samples from clinical and outdoor settings often share properties and similar distribution of bacteria [7], therefore, it is important to study the impact of the air during sandstorms and rising dust on the indoor hospital air. Increasingly high temperatures and hot climates, coupled with high population and over prescription of antibiotic medication, may play a significant role in the presence of

The sand and dust effectively impact human health, the environment, and the economy of countries. Its damage to the infrastructures and interruption to transportation is evident. But the long-term effect of these particles on human health explored here should be studied more [12]. The airborne dust and its particle size determine the amount of impact on people's health. World scientists have linked environmental conditions like dust storms to the increasing pattern of bacterial infections amongst the populations. When the dust particles are inhaled in hot dry weather, the nose and throat mucosa are damaged, giving rise to bacterial infections. This extreme event is prevalent in many parts of the world because of its ability to travel through the earth's atmosphere. However, the main sources of dust are the arid regions of North Africa, the Arabian Peninsula, China, and Central Asia [12]. The Middle East, especially the places like Kuwait and Dubai, experience dusty weather more commonly when compared to others. Kuwait has a subtropical desert climate that results in extremely hot and dry summers with a very short winter. The oil industries present here contribute to toluene and sulphur dioxide pollution. The increase in dust storms every year certainly plays an important role in the antibiotic resistance amongst the people of Kuwait, and it should lead to more studies.

In this study, we were able to isolate the following bacteria from hospital air samples: *Staphylococcus aureus,* coagulase-negative *Staphylococcus*, *Bacillus* spp., *Acinetobacter* spp., *Micrococcus* spp., *Corynebacterium simulans*, *Luteimonas terrae*, *Agrobacterium salinitolerans*, *Corynebacterium amycolatum*, *Corynebacterium casei,* and *Escherichia fergusonii.* Our findings are similar to the study conducted by Toar et al. [13] who studied hospital operating room air samples and found the following isolates: *Klebsiella pneumoniae*, coagulase-negative *Staphylococcus,**[1] and *Bacillus subtilis*. In another study Solomon et al. [14] collected hospital indoor air samples via passive air sampling method. They found coagulase-negative *Staphylococci* (29.6%), *Staphylococcus aureus* (26.3%), *Pseudomonas aeruginosa* (5.3%), *Acinetobacter* spp., (9.5%), *Enterococci* species, *Enterococcus faecalis* and *Enterococcus faecium* (16.5%), *Acinetobacter* species (9.5%), and *Escherichia coli* (5.8%). Similar types of bacteria were found in our study, however, only they did not isolate *Bacillus* spp., and we did not isolate *Enterococcus* spp. In another study by Kunwar et al.[15] across eight hospitals in Kathmandu, Nepal, isolated bacteria from hospital air

samples included *Staphylococcus aureus* (47.18%), *Pseudomonas* spp. (1.82%), and others such as coagulase negative *Staphylococcus, Streptococcus* spp., *Micrococcus* spp., *Bacillus* spp., *E. coli,* and *Proteus* spp. Like our study, Kunwar et al. were able to isolate *Staphylococcus* spp. and *Bacillus* spp., but not *Acinetobacter*.

In terms of *Staphylococcus*, the isolates in our study exhibited resistance to trimethoprim, erythromycin, ciprofloxacin, cefoxitin, tetracycline, and clindamycin; only 12% were resistant to oxacillin. In the study performed by Solomon *et al*. [14], methicillin resistance was observed in 38.9% of the isolates, higher than this study. In other studies performed by Toar et al. [13] and Kunwar et al. [15], the results of antibiotic sensitivity testing for their isolates were not reported. Solomon et al. [14] found that *Acinetobacter* were resistant to gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin; whereas, in our study, we found that *Acinetobacter* were resistant to imipenem, meropenem, and tetracycline. Our finding of multidrug resistance pattern for *Acinetobacter* was different from Solomon et al. In another study conducted by Shamsizadeh et al. [16], *Acinetobacter* resistant to ceftazidime, imipenem, and gentamicin were isolated from the intensive care units.

Outdoor Air Isolates

We isolated *Pseudomonas*, *Staphylococcus aureus,* and coagulase-negative *Staphylococcus*, from outdoor air samples which were similar to the findings from studies as discussed below. In one study [17], the dominant species isolated from a school in Nigeria were *Escherichia coli*, *Micrococcus* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus* spp. In another study [18] indoor and outdoor air samples in a school in India were tested and *Micrococcus, Staphylococcus, Streptococcus, Bacillus, Legionella, Pseudomonas, Klebsiella, and Mycobacteria* were isolated from both samples. They observed differences between locational indoor concentrations of the microorganisms depending on which areas were more frequently visited and showed environmental outdoor microorganisms can spread indoors.

Li et al. [19] performed a global survey of antibiotic-resistance genes from urban air and found that there were 30 antibiotic-resistance gene subtypes resistant to the following classes of antibiotics: beta-lactam, quinolones, tetracyclines, macrolides, aminoglycosides, sulphonamides, and vancomycin. Cities that were included in the study were Haikou, Hong Kong, Guangzhou, Shanghai, Beijing in China, Bandung in Indonesia, San Francisco in the USA, Melbourne and Brisbane in Australia, Singapore, Paris and Tours in France, amongst others. This study highlighted the notion that urban air across the globe can contain antibiotic-resistant microorganisms.

In this study, various species of *Staphylococcus* and *Bacillus* with different antimicrobial resistance profiles were found from both outdoor and hospital air samples during sandstorms. Comparing the resistance patterns of the isolates obtained from outdoor air samples with hospital indoor air samples, shows remarkably more *Staphylococcus* isolates obtained from atmospheric outdoor air samples were resistant to oxacillin (95% outdoor vs 12% hospital). Resistance to trimethoprim and erythromycin amongst outdoor and hospital *Staphylococcus* isolates were: 65% outdoor vs 73% hospital and 60% outdoor and 50% hospital, respectively. In terms of multiple drug resistance patterns, 75% of the outdoor isolates versus 42% of hospital isolates were resistant to at least three different classes of antibiotics. However, outdoor isolates did not exhibit resistance to linezolid and vancomycin, whilst one isolate collected from hospital air, was resistant to vancomycin and two isolates were resistant to linezolid. This could translate to a higher occurrence of methicillin-resistant *Staphylococcus* spp. and multidrug-resistant strains in atmospheric outdoor air, nevertheless, the findings support the ubiquity of *Staphylococcus* spp. both in outdoor air and in hospital premises.
In contrast to the results of our study, Tamberkar et al., 2007 [20], showed that *Staphylococcus* were isolated more from the outdoor air samples than from indoor samples. The authors attributed this to shedders as being the sources of the high burden of *Staphylococcus,* which disperse large numbers of Gram-positive cocci into the environment. In the same study, they were also able to isolate *Pseudomonas aeruginosa*, which had a higher concentration in the indoor air than outdoor air. In our present study, we were only able to isolate *Pseudomonas* spp. from hospital air samples.

Oxacillin resistance was detected in *Bacillus* isolates from both outdoor (83%) and hospital (63%) air isolates. There were also higher rates of resistance amongst outdoor air isolates against mupirocin (58% outdoor vs 25% hospital), trimethoprim (58% outdoor vs 38% hospital), and erythromycin (50% outdoor vs 38% hospital). Of the outdoor isolates, 75% showed resistance to more than three antibiotic classes, whilst amongst the hospital isolates, only 38% showed multiple drug resistance. Moreover, three of the outdoor isolates exhibiting multiple drug resistance patterns also were resistant to one or two of the following antibiotics: imipenem, linezolid, and vancomycin. Such findings support the notion of environmental *Bacillus* spp. (usually in soil) as a source of hospital-acquired infection [21] that would be harder to treat with available antibiotics.

From indoor air samples we identified *Staphylococcus Species*, *Bacillus*, *Acinetobacter,* and micrococcus. Outdoor air samples contained *Bacillus*, *Pseudomonas*, and *Staphylococcus*. In comparison, *Bacillus* and *Staphylococcus* were found from both outdoor and hospital air samples. *Acinetobacter*, *Micrococcus,* and *Bacillus* were not found in outdoor samples and indicated the possibility of contamination from outdoor environmental sources.

There was no isolated *Acinetobacter* from outdoor air samples. *Acinetobacter* survives best in water and soil, whereas our research was focused on collection of air samples. *Acinetobacter* can be found on human skin and can survive for long under unconducive settings, thus attributing possible contamination of indoor environment [16].

There are some limitations to our study, the sample collection method included both targeted and non-targeted bacteria. Different growth conditions and rates could affect the distribution of the bacteria in the samples. Overgrowing bacteria could inhibit the growth of the slow-growing bacteria through competition for resources and thus affect the results. The study only used data from two hospitals and two outdoor target sites, making statistical relationships for all hospitals in Kuwait difficult, moreover this is the first study in Kuwait to sample air during sand storms and therefore lack of prior research data limited the scope of the study analysis.

In conclusion, antibiotic-resistance is a public health concern that greatly affects the progression and management of infective diseases. In this study, we have determined the types and the distribution of antibiotics-resistant bacteria present in the Kuwaiti air samples and compared the results with the bacteria present in the clinical settings. Dust rising from sandstorms contribute to the species of bacteria seen in clinical settings in Kuwait. Moreover, some bacteria tend to be able to survive for longer periods and thrive in the clinical settings, such as *Acinetobacter* that has the capability of remaining for prolonged periods in the environment. The exact nature and the extend of how environment plays its contributory role in propagation of antimicrobial resistance is still not clear. More attention should be paid to the environment as a critical contributing factor to link the ecological influences to the propagation of resistant bacteria in air.

*[1] Coagulase-negative *Staphylococcus* include *S. saprophyticus, S. epidermidis, S. hominis*

List Of Abbreviation

CCU	Cardiac Care Unit
CFU	Colony-Forming Units
CICU	Cardiac Intensive Care Unit
DNA	Deoxyribonucleic Acid
HEPA	High-Efficiency Particulate Air
ICU	Intensive Care Unit
MIC	Minimum Inhibitory Concentration
PCR	Polymerase Chain Reaction
R2A	Reasoner's 2A Agar
RNA	Ribonucleic Acid
Spp	Species,
WHO	World Health Organization

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article

Conflicts of Interest

No potential conflict of interest was reported by the authors.

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Author contribution

Sara Shamsah: Conceptualization, Methodology, Validation, Analysis, Investigation, Data Curation, and Writing,

Leila Vali: Methodology, Validation Analysis, Investigation, Data Curation, Writing – Review and Editing, Review, Supervision, and Editing.

Dana Al-Kayyalli: Analysis, Data Curation, Review and Editing.
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Figures

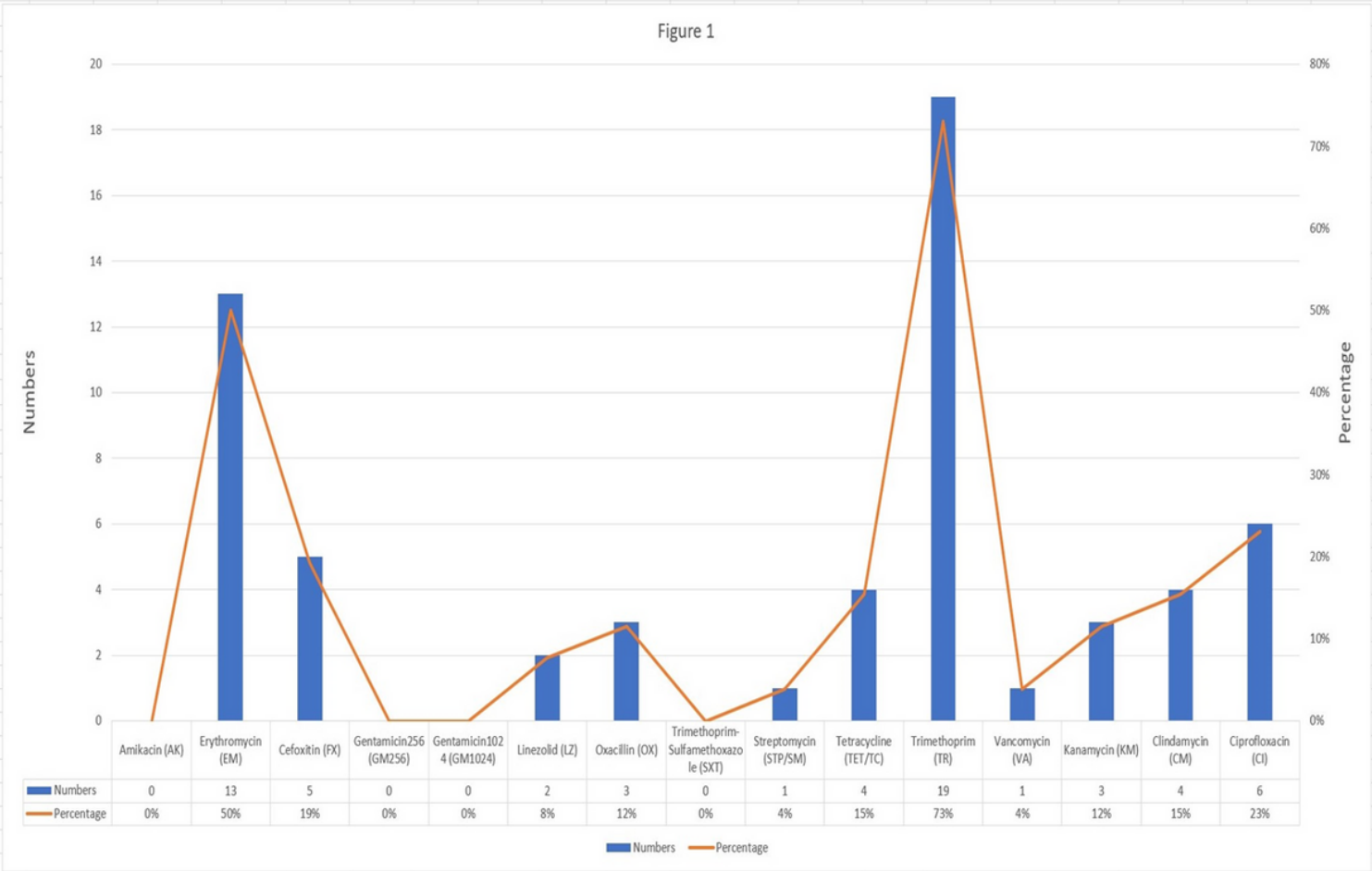

Figure 1

The graph in (Figure.1) shows the overall resistance patterns of Staphylococcus species obtained and cultured from air against medically important antibiotics used in clinical settings.

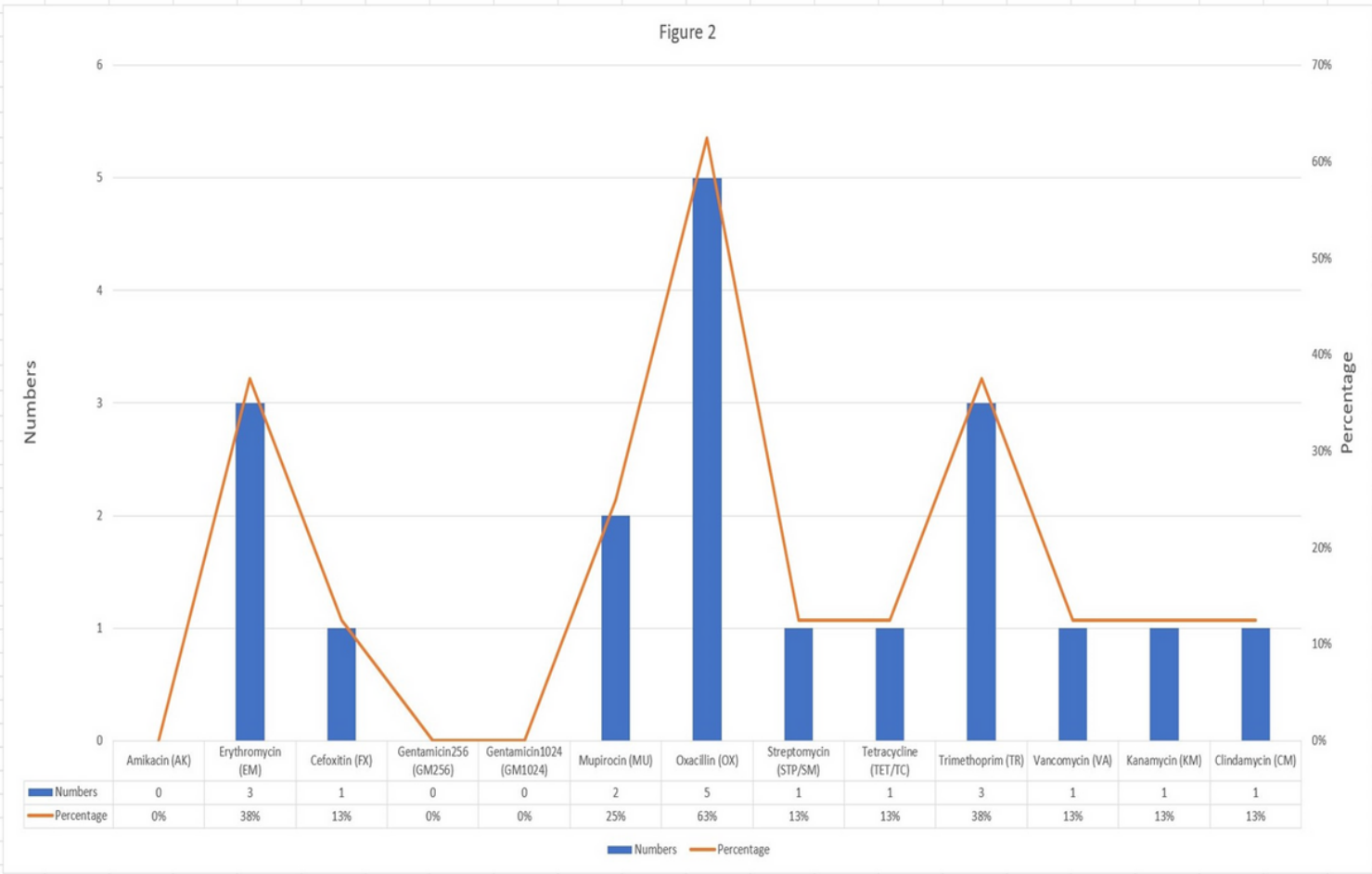

Figure 2

25% (n=)were resistant to mupirocin, whilst the following antibiotics were found to have one (13%) isolate to which it was resistant to:cefoxitin, streptomycin, tetracycline, vancomycin, kanamycin, and clindamycin (Figure.2).

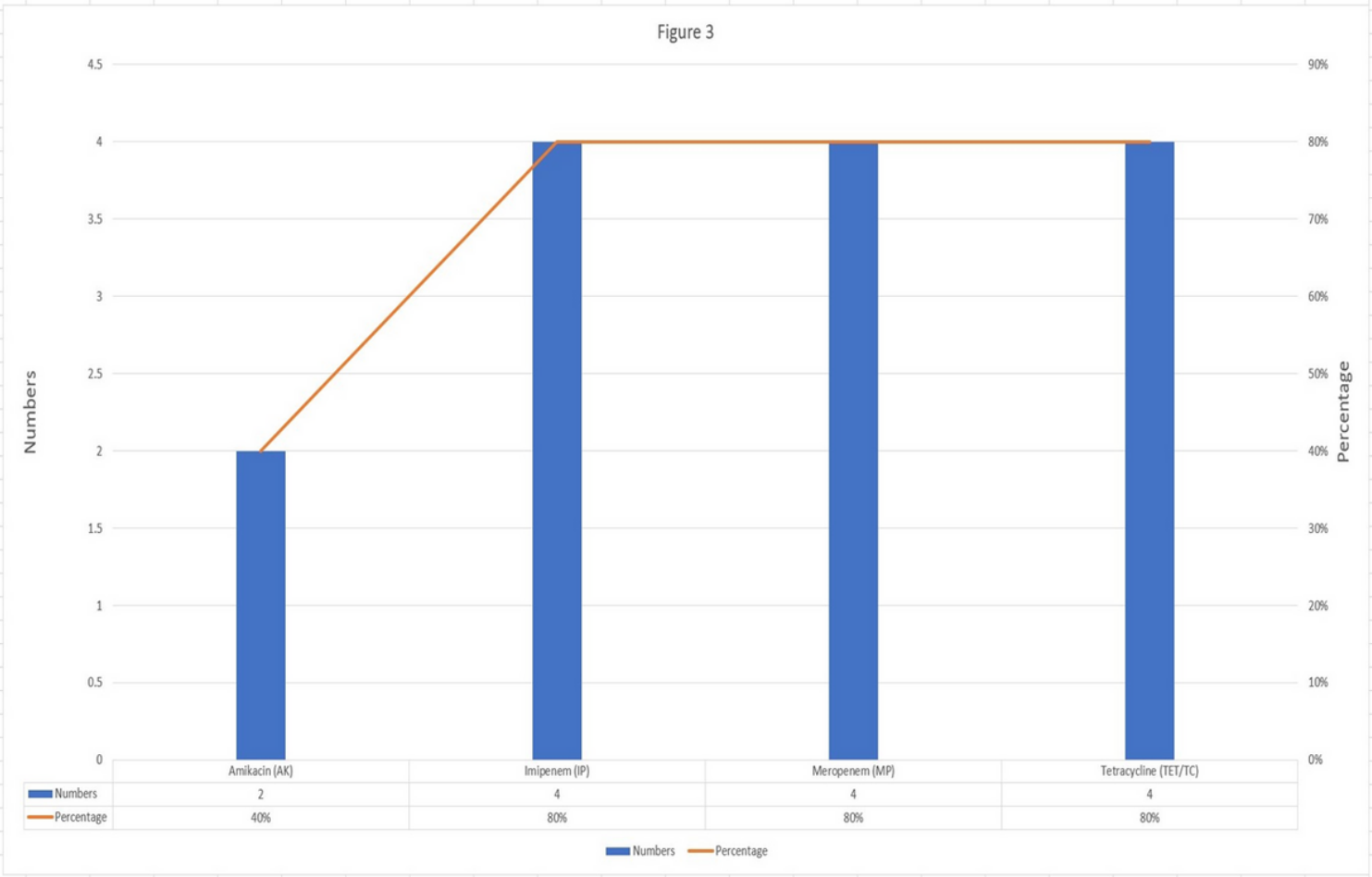

Figure 3

There were five isolates of Acinetobacter cultured from hospital air, four of which were A. baumanii, whilst the other one was A. lwoffii . 80%(n=) of A. baumanii were resistant to imipinem, meropenem, and tetracycline while 40% (n=1) were resistant to amikacin (Figure.3).

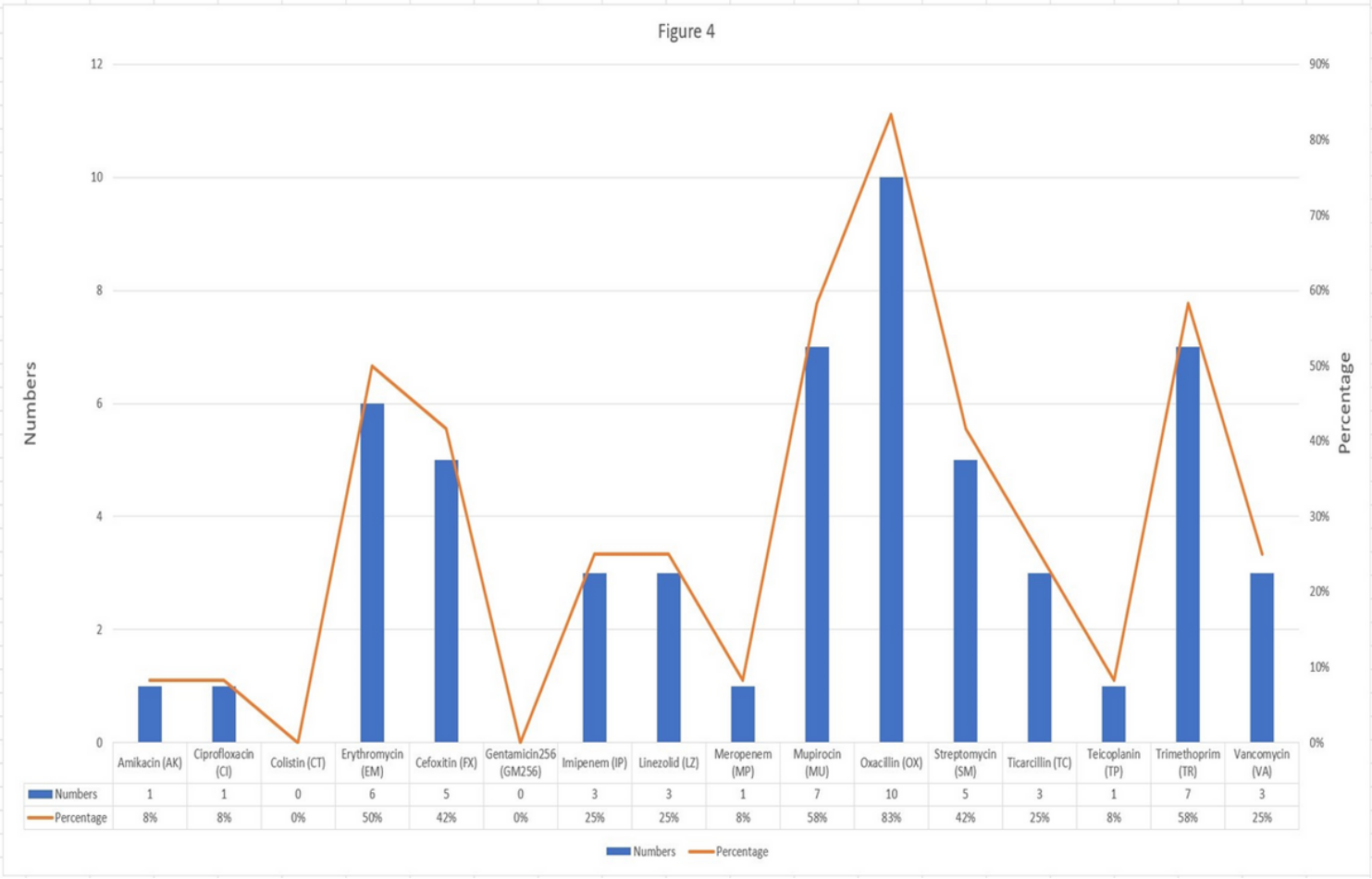

Figure 4

Both Solibacillus isronensis B3W22 isolates showed resistance to erythromycin, cefoxitin, imipenem, mupirocin, oxacillin, streptomycin, tetracycline, and trimethoprim (Figure.4).

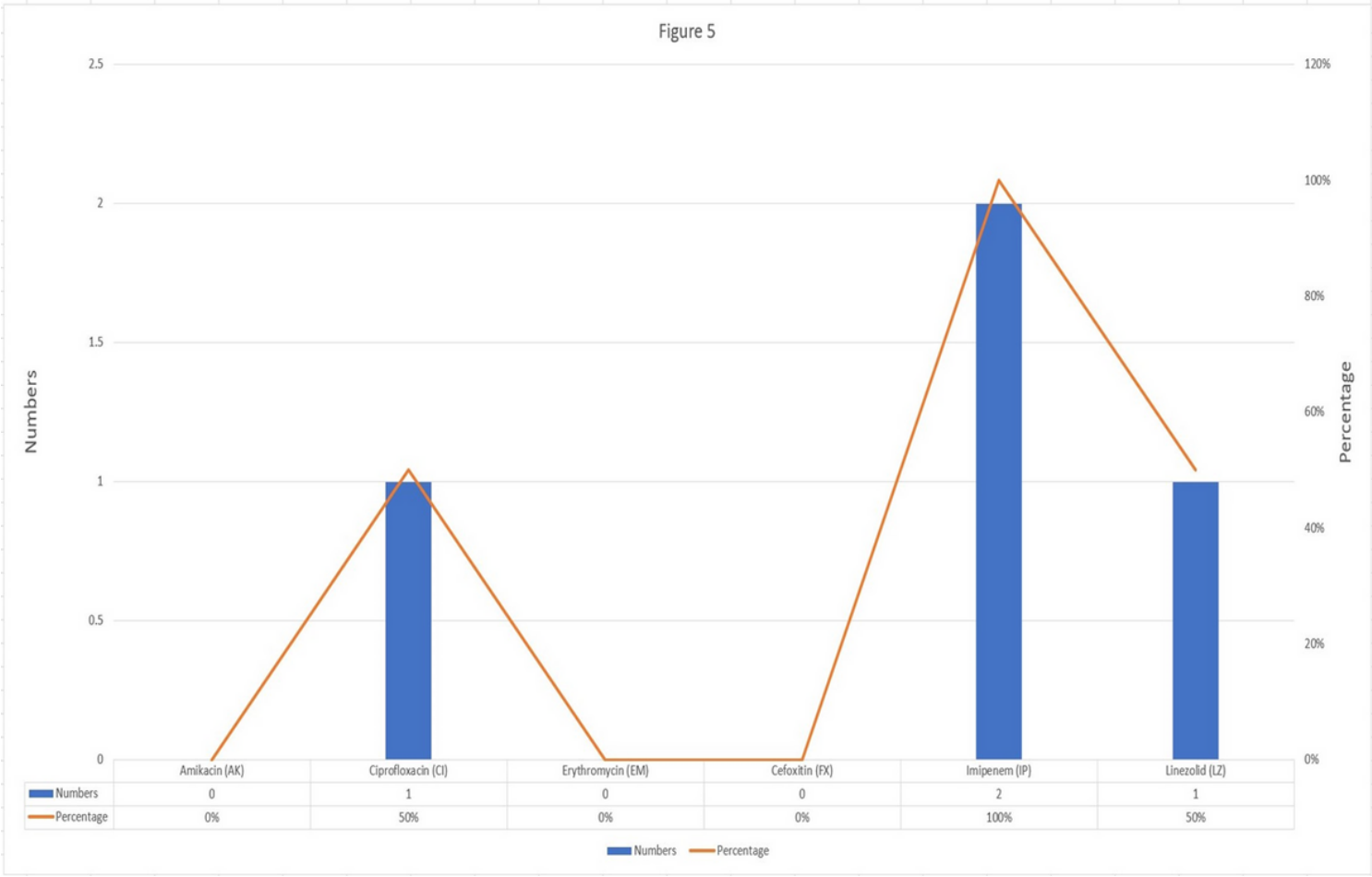

Figure 5

Two isolates of Pseudomonas spp. were obtained consisting of P. xanthomarina and P. xiamenensis DSM22326. Both showed resistance to Imipenem. P. xiamenensis exhibited multiple drug resistance pattern, including resistance to ciprofloxacin, linezolid and imipenem (Table 6) (Figure 5).

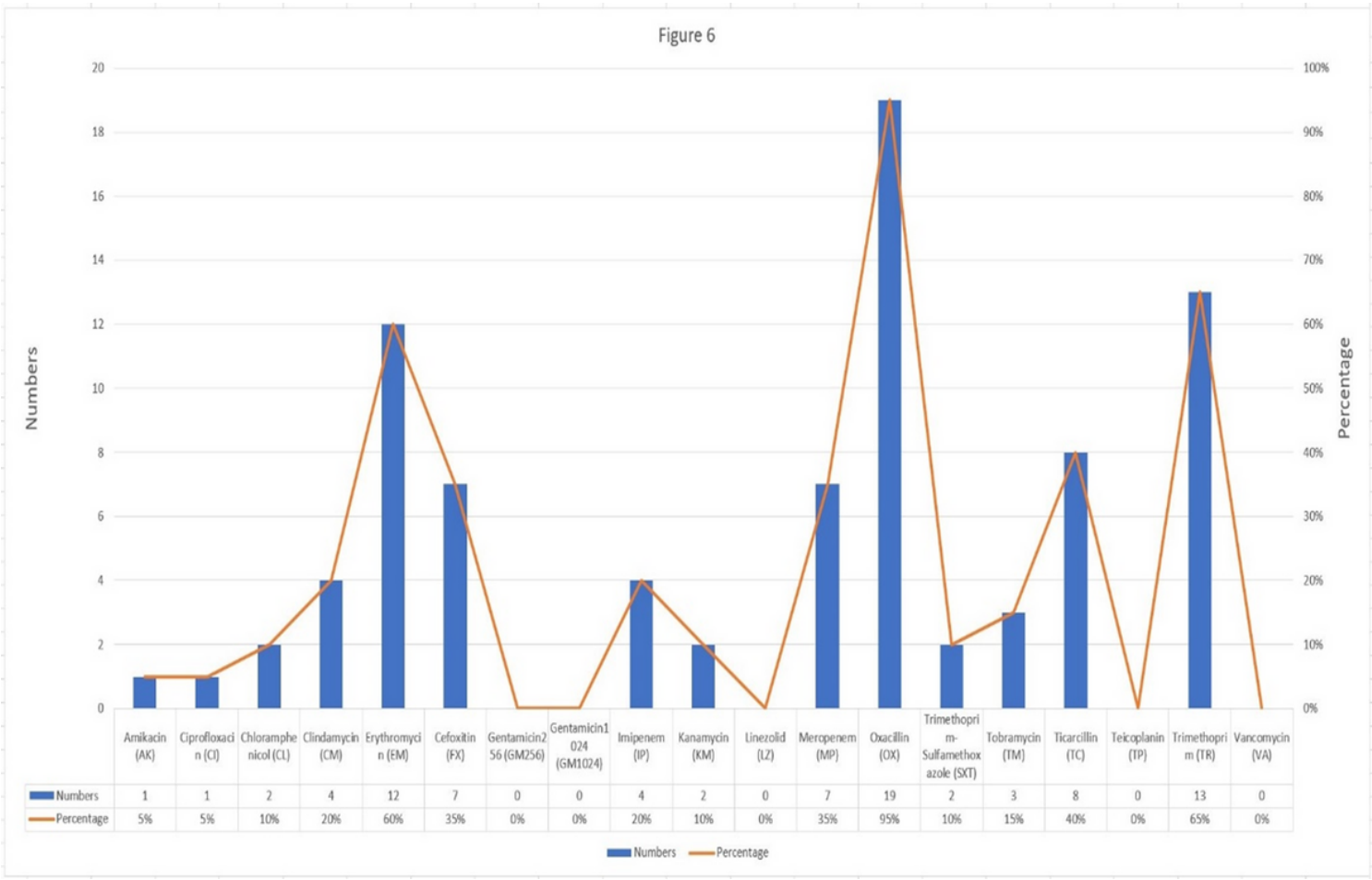

Figure 6

Additionally, there were 10% (2 out of 20) resistance to each of chloramphenicol, kanamycin, sulfamethoxazole-trimethoprim, and 5% (1 out of 20) resistance to each of amikacin and ciprofloxacin (Figure 6).