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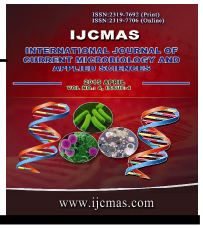

Original Research Article

Comparative Antimicrobial Susceptibility Profiling of Tigecycline and Other Antibiotics against Clinical and Environmental Isolates

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Keywords

Tigecycline, *in vitro* Susceptibility, Resistance, Clinical Environment

A B S T R A C T

The study of the irrational extensive use of antibiotics has led to an increase in the rate of resistant strains of bacteria. Likewise, the study of new antibiotic agents is very critical. In this manner tigecycline was studied in this research survey. The activity of 11 antibiotics against 264 pathogens isolated from clinical specimen and 225 pathogens isolated from environmental samples was evaluated. The comparison of similar strains reveals higher total resistance to the environmental ecosystem than to the clinical ones. Study groups included *Escherichia coli*, *Enterobacter spp.*, *Enterococcus faecium* and other *Enteroccoccus spp*., *Klebsiella oxytoca* and other *Klebsiella spp*., *Pseudomonas spp.*, and *Staphylococcus aureus*. Isolates tested by the broth microdilution method under common concentrations and the results interpreted according to the CLSI criteria. Up to 38% of environmental *E. coli* was resistant to amoxycillin/clavulanic acid, 65% of *S. aureus* to levofloxacin, 39% of *Klebsiella spp*., and 33% of *Klebsiella oxytoca* to ceftriaxone, 50% of environmental *E. coli*, 35% of *Enterobacter spp.*, and 23% of *Pseudomonas spp*., shown resistance against cefepime. Resistant to tigecycline was the 27% of *E. coli*, 8% of *Enterococcus spp.*, and 62% of *S. aureus*, all isolated from the environment. Our results indicate a certain dispersion of antibiotic resistance into environment.The comparison of similar strains reveals significantly higher total resistance to the environmental ecosystem than to the clinical ones. As far as tigecycline is concerned, it is obvious that there is a rapid spread of new antibiotics in the environment, which is granted during the last seven years in Greece only in clinical practice.

Introduction

Irrational and extensive use of antimicrobial agents has led to an increase in the rate of resistant strains of bacteria whether they come from the environment or from clinical sources associated with human infections (Baquero *et al.,* 2008). Increasing resistance rates result in a subsequent reduction of antimicrobial efficacy due to the decreased activity of some agents, depending on the target of the agent. This often leads to higher numbers of nosocomial infections, associated with resistant bacterial species commonly associated with certain types of infections, as well as to a number of infections occurring from unusual or non-pathogenic bacteria (Livermore *et al.,* 2001). Antibiotic resistance in bacteria may be an intrinsic trait, or it may be acquired. More specifically, genes which are responsible for antibiotic resistance are usually located on the chromosomes of the environmental strains (D'Acosta *et al.,* 2006; Fajardo *et al.,* 2008). In recent surveys, it has been established that these genes also exist in plasmids that can easily be transferred to human pathogens (Baquero *et al.,* 2008). The majority of antibiotics used in veterinary practice for the treatment of animal infections as well as growth factors included in animal feed additives, which are partially metabolized, excreted and absorbed into plant tissues and aquatic environments (Dolliver *et al.,* 2008). The risk to public health occurs from consumption of the tissues from these animals as food sources for humans, who may acquire resistant strains and potentially pass these into the environments of healthcare facilities or the community. Molecular studies have shown that resistant bacteria are transferred from food to humans *via* the food chain, after handling or consuming meat or byproducts that have been infected (Lateef *et al.,* 2005). Antibiotics are also used in aquaculture and agriculture in order to increase the productivity of animals and plants.

Likewise, the need for the discovery of new antimicrobial agents is very critical. Tigecycline is a relatively recent antibiotic that was developed as a broad-spectrum antimicrobial agent that can be used for the treatment of numerous Gram-negative and Gram-positive bacteria.

Tigecycline is approved against skin and soft tissue infections and complicated intra-abdominal infections caused by *Enterococci*, *Staphylococci*, *Streptococci* and enterobacteriaceae (Castanheira *et al.,* 2008). Tigecycline was approved on April 2005 by the FDA and in the European Union (EU) in April 2006 (Tygacil Package Insert, 2010). In the last five years, in which tigecycline was commercialized; many surveys were conducted and published in the International literature. The outcome of the results was quite promising in most cases, as far as clinical isolates are concerned (Nannini *et al.,* 2003; Yin *et al.,* 2005). However, the international literature on the application of tigecycline on environmental strains has not yet been determined. In addition, comparative studies between similar bacterial species for comparing antimicrobial resistance issues among environmental and clinically derived strains are also not well established.

Likewise, in the frame of this research, various data are presented through microbiological testing of various antibiotics among environmental strains isolated from Evros regions in Greece and clinical strains collected from various Greek hospitals. The main considerations and emphases of this investigation were; to compare the resistance of common categories of antimicrobial factors between environmental and clinical strains; a subject that the

International and Greek literature is lacking. Secondly, the comparative assessment of resistance to tigecycline against other antibiotics on environmental and clinical isolates will be determined.

Materials and Methods

Sampling

A total number of 264 clinical isolates and 225 respective isolates from aquatic environment, mainly surface water, were examined, and compared for their susceptibility against 11 antibiotics, included tigecycline. The clinical isolates were collected from hospitals located in Greece, in a period of 18 months. Patients were hospitalized with intra-abdominal, respiratory, urinary and skin and soft tissue infections.

Environmental isolates were collected from the rivers of Evros and Erythropotamos located in the North-East region of Greece. These rivers receive a large amount of domestic and agricultural effluents in forms of treated wastewater or runoff waste from farming and livestock activities.

Clinical isolates, already identified within the corresponding microbiology departments, were transferred to the laboratory and stored in the freezer in glycerol broth (30%). Environmental samples were analyzed by the filtration method in nitro-cellulose filters with a 0.5μm pore diameter. Filters were placed on the surface of various media and incubated accordingly. Identification completed by using commercial test kits (API, bioMerieux, Marcy l'Etoile, France) or chromogenic media. In addition, some of the strains were further identified by using the Vitek 2 automated system (bioMerieux, Marcy l'Etoile, France), according to the instructions given by the manufacturer.

Susceptibly testing

Minimum Inhibitory Concentrations (MICs) of the isolates were determined using the microdilution method. Initially, the organisms were inoculated in 5ml of sterilized demineralized water. The inoculum suspension was vortexed for 5 to 10 seconds and was standardized to a 0.5 McFarland standard. 10μl of inoculum was pipetted and transferred to 11ml of sterilized Cation Adjusted Mueller-Hinton Broth. Then the inoculum was inverted for 8 to 10 times and pipetted (100 μl in each well) in commercial 96 well microtitre plates filled with different concentrations of dehydrated antibiotics by using a multi channel pipettor. A sealer was placed over the plate and the panel was incubated in the incubator for approximately 24h at 37^0C.

The panels were read using a TREK viewer and the results were recorded directly to a planned susceptibility report form. The antibiotics and their respective low and high concentrations are presented in Table 1.

Quality control measures were utilized by testing *S. pneumoniae* ATCC 49619, *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Categorical interpretation of MIC values was performed according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI document, 2011).

Results and Discussion

Susceptibility results with MIC_{50} and MIC_{90} for both clinical and environmental strains are shown in Table 2. Interpretations

according to the CLSI criteria (when applicable) and percentages of sensitive, intermediate or resistant strains are shown in Table 3.

Up to 38% of the environmental *E. coli* strains were resistant to amoxicillin/clavulanic acid ($MIC_{50/90}$, 2/32 mg/L), 65% of *S. aureus* to levofloxacin ($MIC_{50/90}$, 4/32 mg/L), 39% of *Klebsiella spp*. and 33% of *Klebsiella oxytoca*, to ceftriaxone ($MIC_{50/90}$, 16/64 mg/L), 50% of environmental *E. coli* ($MIC_{50/90}$, 2/32 mg/L), 35% of *Enterobacter spp.* ($MIC_{50/90}$, 16/32 mg/L) and 23% of *Pseudomonas spp.*, ($MIC_{50/90}$, 0.5/32 mg/L), shown resistance against cefepime. Resistance to ampicillin observed among environmental strains was 72%, 85% and 28% for *E. coli* ($MIC_{50/90}$, 32/32 mg/L), *Enterobacter spp.*, ($MIC_{50/90}$, 32/32 mg/L) and *Klebsiella oxytoca* ($MIC_{50/90}$, 0.5/32 mg/L), respectively. The majority of clinical (83%) and environmental (65%) strains of *S. aureus* ($MIC_{50/90}$, 4/16 and 1/4mg/L respectively). Amikacin, resistant rates were 32% among the *E. coli* environmental strains ($MIC_{50/90}$, 1/64 mg/L), 28% of *Klebsiella spp.*, ($MIC_{50/90}$, 32/64 mg/L) and 20% of *Pseudomonas spp.* ($MIC_{50/90}$, 1/64 mg/L). Ceftazidime resistance was 35% among *Enterobacter spp.*, ($MIC_{50/90}$, 8/32 mg/L), 78%, *Klebsiella oxytoca* ($MIC_{50/90}$, 32/32 mg/L) and 83% of *Klebsiella spp.*, ($MIC_{50/90}$, 32/32 mg/L). Resistance to tigecycline was 22% among *E. coli* ($MIC_{50/90}$, 0.5/8 mg/L), 8% of *Enterococcus spp.*, ($MIC_{50/90}$, 0.5/1 mg/L) and 62% of *S. aureus* ($MIC_{50/90}$, 1/16 mg/L), all isolated from the environment. Finally, most of the strains, regardless of their origin, were sensitive to meropenem except a 5% of clinical *S. aureus* ($MIC_{50/90}$, 0.12/2 mg/L) exhibiting resistance.

Overall a variable prevalence of resistant strains was observed mainly from those of environmental origin in contrast to those isolated from the clinical environment (Figure 1). Among them, *E. coli* environmental strains were resistant of up to seven antibiotics (5 general antibiotic categories: penicillins, quinolones, cephalosporins, aminoglycocides & tetracyclines) in percentages varying from 10 to 68% (Fig. 1a). *Enterobacter spp.*, were resistant to five antibiotics (3 general antibiotic categories: penicillins, cephalosporins & aminoglycocides) (Fig. 1b), *Klebsiella oxytoca* (Fig. 1c) and other *Klebsiella spp.*, (Fig. 1d) to five antibiotics (3 general antibiotic categories: penicillins, cephalosporins & aminoglycocides), *Pseudomonas spp.* (Fig. 1e) in three antibiotics (3 general antibiotic categories: quinolones, cephalosporins & aminoglycocides), *S. aureus* in five antibiotics (4 general antibiotic categories: quinolones, cephalosporins, penicillins & tetracyclines) (Fig. 1f) and *Enterococcus spp.*, in one (Fig. 1g). *Enterococcus faecium* was susceptible to all antibiotics.

Sixteen *E. coli* environmental strains were isolated from surface waters and 32 respective strains were collected from Greek hospitals. The lowest MIC values observed were ceftriaxone and meropenem (0.06 μg/mL) followed by tigecycline (0.5 μg/mL), in the case of environmental isolates. In clinical isolates, tigecycline was the most effective antibiotic with MIC value 0.06 μg/mL. The increasing resistance of *E. coli* clinical strains could probably be explained by the presence of the gene encoding the CTX-M-15, an extended-spectrum-b-lactamase enzyme, which confers multiresistance to antibiotics (Ruppe *et al.*, 2009). The above agree with results from our previous study (Mantzourani *et al.*, 2011) where resistance to beta-lactams and cephalosporins was exhibited and may be

explained by a common mechanism of antibiotic resistance (AmpC). The high resistance of environmental *E. coli* isolates could be interpreted by the production of beta-lactamases (Lateef *et al.,* 2005). Similar results occurred in the present study as well. References about beta lactamase gene transfer between various bacterial species which previously did not have such capacity were made in the past as well (Mascaretti *et al.,* 1999; Kawakami *et al.* 2000; Silva *et al.,* 2000). Environmental isolates resistant to tigecycline suggests dispersion of this new antibiotic, which is exclusively used in humans, may have spread into the environment.

In clinical strains of *Enterobacter spp*, the lowest MIC value observed was for tigecycline (0.25 μg/mL) compared to 0.06 μg/mL in environmental strains. In the case of environmental *Enterobacter spp* strains, ceftriaxone and meropenem also had low MIC values (0.06 and 0.06 μg/mL). According to a recent survey that took place in the General Hospital of Alexandroupolis (Panopoulou *et al.,* 2010), isolates of *E. cloacae* were examined and three kinds of genes were detected; the bla_{vim-12} gene, the bla_{Amp-C} gene and the bla_{vim-1} gene, providing strains with resistance to b-lactams, aminoglycosides and trimethoprim. These results are in accordance with our survey. Regarding environmental strains of *Enterobacter spp*, strains isolated from the aquatic ecosystem were shown resistant to 3 antibiotics, while another 5 *Enterobacter spp* strains were found having resistance to 4 additional antibiotics (erythromycin, tetracycline, cotrimoxazole and chloramphenicol) (Lateef *et al.,* 2005). Resistance to at least 6 out of 7 antibiotics is interpreted as multiple resistance and is mainly due to plasmid transfer (Zuccato *et al.,* 2010). It is noteworthy that 40% of *Enterobacter spp* isolated from sewage and rivers show resistance to the beta-lactamases (Li *et al.,* 2010) and this is due to the presence of plasmids. According to our results during similar studies (Mantzourani *et al.,* 2011) resistance to beta-lactamases, aminoglycosides and cephalosporins comes in agreement with the international literature (Hirsch *et al.,* 1999; Lateef *et al.,* 2005).

The lowest MIC value, in the case of clinical isolates of *Klebsiella spp* was exhibited by meropenem (0.06 μg/mL), followed by levofloxacin (0.12 μg/mL) and finally by tigecycline (0.25 μg/mL). In the case of environmental *Klebsiella spp*, tigecycline was the most effective antibiotic (0.06 μg/mL) in the total of isolates (100% susceptible) and the next most effective antibiotic was amoxicillin (0.12 μg/mL) but in the majority of the isolates (94%). Susceptibility to levofloxacin and meropenem was observable by a MIC_{50} value of 0.06 μg/mL, followed by tigecycline with a MIC value of 0.25 μg/mL in clinical strains. Comparing the respective results in environmental strains the lowest MIC values were shown for tigecycline (0.06 μg/mL) and the next most effective antibiotic was amoxicillin (0.25 μg/mL). Moreover, according to our results, environmental *Klebsiella spp*. showed resistance to beta-lactams, penicillins, fluoroquinolones and cephalosporins. These results are consistent with literature although there are insufficient references for the environmental ecosystem (Levesque *et al.,* 1995; Livermore *et al.,* 2001). *Pseudomonas spp.* strains were isolated from both sources; environmental and clinical. Susceptibility observed to clinical strains reached 95 % in the case of meropenem (0.25 μg/mL) followed by a MIC value of 0.5 μg/mL for tigecycline compared with a susceptibility of 73% for ceftriaxone (0.06 μg/mL) and a MIC value of 0.5 μg/mL for tigecycline for the environmental ones.

Pseudomonas spp. resistance is developed somewhat different compared to that demonstrated in *Enterobacteriaceae* towards acquired or chromosomal beta-lactamases. According to the results presented in this study, *Pseudomonas spp.* was found resistant to cephalosporins, aminoglycosides, penicillins, and fluoroquinolones (Mantzourani *et al.* 2011). For the first 2 groups of antibiotics, the outcome is in accordance with the literature (Hancock, 2005). However, regarding the last 2 groups it could not be found comparable results and this issue needs further study. Antibiotic resistance observed in the case of *Enterobacteriaceae,* could be possibly explained by mechanisms of antibiotic resistance common with the aforementioned strains, as beta-lactams and cephalosporins.

Regarding the study of Gram-positive bacteria, *Enterococcus faecium* isolates from environmental source exhibited high susceptibility to tigecycline (0.06 μg/mL) in their total, followed by minocycline and amoxicillin (0.5 μg/mL). The clinical isolates of *Enterococcus faecium* showed high susceptibility to tigecycline in their total (0.03 μg/mL) and a number of four other antibiotic followed this MIC value (amoxicillin, levofloxacin, ceftriaxone & ampicillin with 0.12 μg/mL).

In the case of *Enterococcus spp.* isolates originated from environmental source, tigecycline was quite effective along with levofloxacin, amikacin & minocycline (0.5 μg/mL). Regarding the isolates of clinical origin, tigecycline exhibited by far the lowest MIC_{50} value in comparison with the other antibiotic agents (amoxicillin, levofloxacin, ceftriaxone & ampicillin with MIC_{50}: 0.03 μg/mL). These results are in accordance with other trials, since in one survey in Portugal from 1996 to 2008, 1140 *Enterococci* were collected from humans, food products, animals and the environment. From these strains only 10 exhibited resistance to tigecycline while all the others were susceptible (Freitas *et al.,* 2011).

Finally, *Staphylococcus aureus* isolates from environmental source showed high susceptibility to meropenem (0.25 μg/mL) while tigecycline had an MIC_{50} value equal to 1 μg/mL. However, *Staphylococcus aureus* strains isolated from clinical source showed high susceptibility primarily to tigecycline, levofloxacin and meropenem (0.12 μg/mL) and secondarily to minocycline (0.25 μg/mL). Similar results were found in a comparative study on environmental and clinical isolates of *S.aureus* that took place in Cameroon and the Netherlands (Nkwelang *et al.,* 2009). In these studies vancomycin was the most effective antibiotic agent against *S.aureus* and a correlation between tetracycline resistance determinants tet(K) or tet(M) is implied since tetracycline-susceptible isolates were more often susceptible to tigecycline (Verkade *et al.,* 2010).

The comparison of similar strains reveals significantly higher total resistance to the environmental ecosystem than to the clinical ones. *Klebsiella oxytoca* and *Pseudomonas spp.* are inhabitants of the environmental ecosystems; soil, waters, plants and their foliage and they became opportunistic pathogens for humans. Specific adaptation to their natural environment and possible mutations seem to arm these strains against the development of antibiotic resistance.

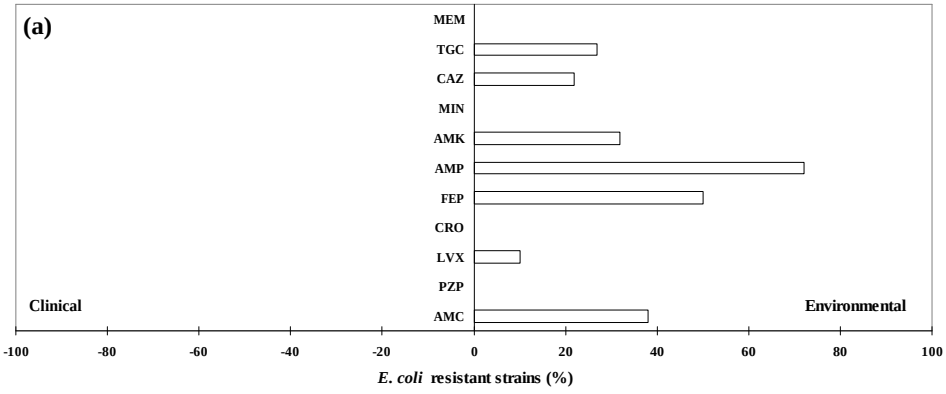
The environmental strains were isolated from Evros River in Greece, which flows down from Bulgaria and Turkey, and probably has received semi-urban, rural and industrial wastes (Vavias *et al.,* 2011). Additionally this same region has several breeding animal farms with immediate risks from irrational use of antibiotics in animals.

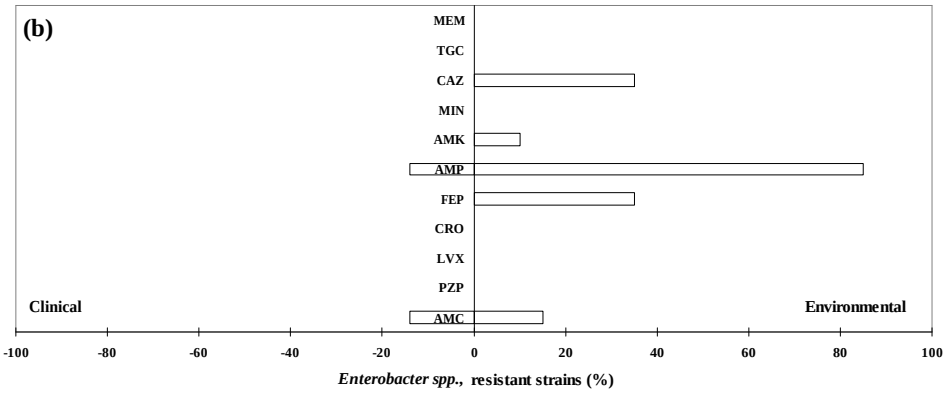
As far as tigecycline is concerned, it is obvious that there is a rapid spread of new antibiotics in the environment, which is granted during the last seven years in our country only in clinical practice.

Table.1 Antibiotics used for the present study, in their respective concentrations

Antibiotic	Low concentration (μg/ml)	High concentration (μg/ml)
Amoxycillin+clavulanic acid	0.06-0.12	16-32
Piperacillin/tazobactan	0.06/4 – 0.12/4	0.25/4 – 128/4
Levofloxacin	0.008-0.015	0.03-8
Ceftriaxone	0.06-0.25	0.5-64
Cefepime	0.5-4	8-32
Ampicillin	0.5-32	
Amikacin	0.5-1	2-64
Minocycline	0.5-16	
Ceftazidime	8-32	
Tigecycline	0.008-2	4-16
Meropenem	0.06-16	

Figure.1 Resistance to various antibiotics from clinical and environmental strains

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AMK
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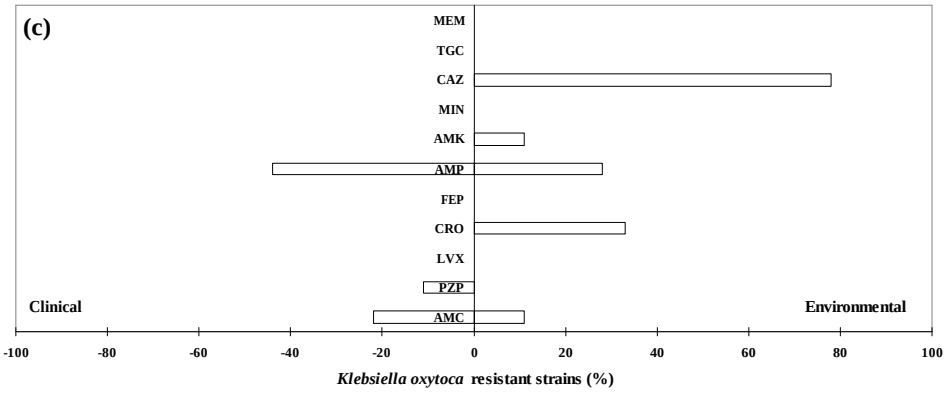
Enterobacter spp., resistant strains (%)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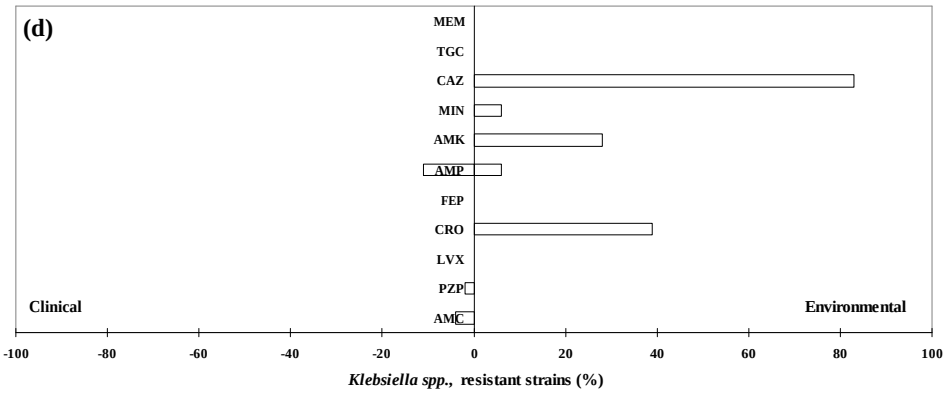
Klebsiella oxytoca resistant strains (%)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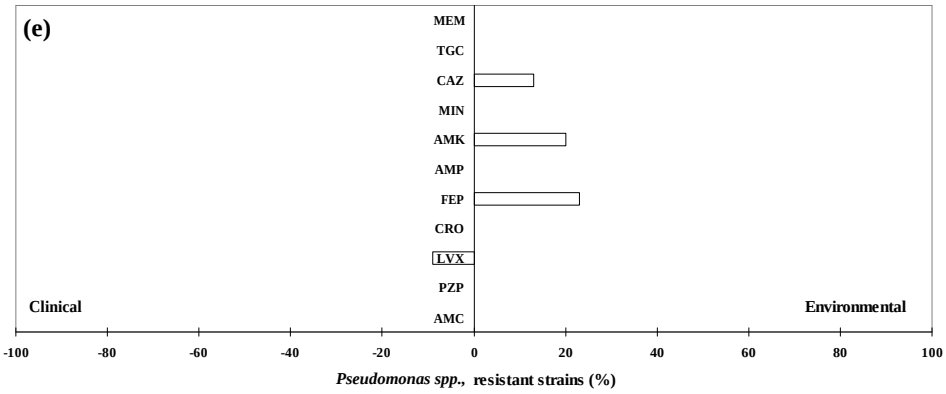
Klebsiella spp., resistant strains (%)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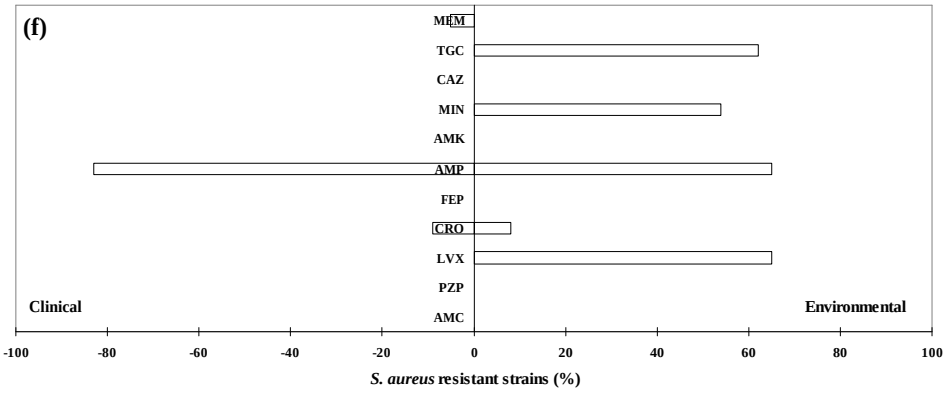
Pseudomonas spp., resistant strains (%)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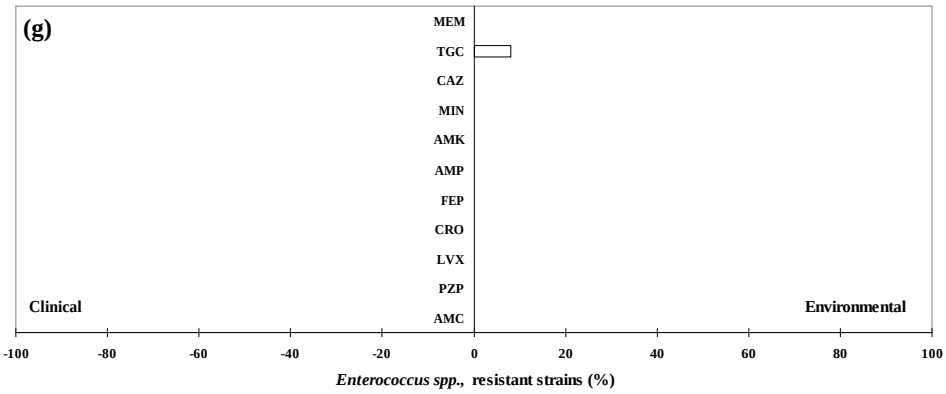
S. aureus resistant strains (%)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Clinical
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-100 -80 -60 -40 -20 0 20 40 60 80 100
Enterococcus spp., resistant strains (%)

Table.2 Values of MIC50 and MIC90 for 11 antibiotics tested in clinical (n=264) and environmental (n=225) strains

Strain	*	N	AMC		TZP		LVX		CRO		FEP		AMP		AMK		MIN		CAZ		TGC		MEM	
			MIC_5	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC
Pseudomonas spp.	E	28	0.12	2.5	2	2	0.5	1	16	64	8	32	0.5	8	32	64	0.5	2	32	32	0.06	0.50	2	2
	C	47	16	16	2	16	0.25	4	4	32	1	16	16	16	1	32	8	8	8	16	0.5	4	0.25	4
S. aureus *E. coli*	E	30	16	32	1	64	0.12	2	0.06	16	0.5	32	32	32	1	64	2	16	8	32	0.5	1	0.25	1
	C	60	4	8	0.5	8	0.015	4	0.06	16	0.5	4	2	16	1	32	1	8	8	16	0.06	0.25	0.06	0.25
	C	33	1	8	1	16	0.12	4	4	32	4	16	16	32	1	64	0.25	8	8	32	0.12	0.12	0.12	2
	E	60	2	32	1	2	0.12	1	0.06	16	2	32	32	32	1	64	0.5	4	8	32	0.5	8	0.06	1.0
	E	27	0.5	2	0.5	4	4	32	4	16	4	8	1	4	0.5	32	16	16	8	8	1	16	0.25	1
Enterobacter spp.	C	31	16	32	0.25	64	0.03	4	0.06	32	0.5	8	2	32	0.5	8	1	8	8	8	0.06	1	0.06	2
	E	20	16	32	4	15.2	0.5	1	16.	32	16	32	32	32	0.5	57	2	3.8	8	32	0.25	1.0	2	2
Enterococcus faecium	C	14	0.12	0.5	0.5	2	0.12	0.25	0.12	0.12	-	-	0.12	0.5	-	-	0.25	1	-	-	0.03	0.03	0.25	0.5
	E	17	32	32	8	8	1	1	4	16	16	32	32	32	0.5	1.0	0.5	2	8	32	0.06	0.25	2	2
Enterococcus spp.	C	28	0.12	0.5	8	8	0.12	0.25	0.12	0.12	-	-	0.12	0.5	-	-	4	4	-	-	0.03	0.03	0.25	0.5
	E	25	16	32	4	16	0.5	0.5	16	32	1	32	32	32	0.5	64	2	4	8	32	0.5	1	2	4
Klebsiella oxytoca	C	19	4	32	1	128	0.06	4	0.12	32	0.5	16	16	32	4	16	1	4	8	16	0.25	1	0.06	8
	E	18	0.25	32	2	2	1	1	16	64	16	32	0.5	32	16	64	0.5	2	32	32	0.06	0.25	2	2
Klebsiella spp.	C	32	8	16	4	64	0.12	4	1	32	1	16	16	32	2	32	2	8	8	16	0.25	1	0.06	8

*C: clinical, E: Environmental

AMC: Amoxycillin/Clavulanic acid, TZP: Piperacillin/Tazobactam, LVX: Levofloxacin, CRO: Ceftriaxone, FEP: Cefepime, AMP: Ampicillin, AMK: Amikacin,

MIN: Minocycline, CAZ: Ceftazidime, TGC: Tigecycline, MEM: Meropenem

Table.3 Interpretation of susceptibility results (%) in pathogens isolated from clinical (n=264) and environmental (n=225) sources

Strain	*	N	AMC			TZP			LVX			CRO			FEP			AMP			AMK			MIN			CAZ			TGC			MEM		
			S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
E. coli	C	60	92	8		94	6		81	19		98	11		97	3		72	28		86	14		89	11		86	14		100			100		
	E	60	62		38	100			90		10	88	12		50		50	28		72	68		32	90	10		78		22	73		27	92	8	
Enterobacter spp.	C	31	36	50	14	86	14		86	14		86	14		93	7		64	22	14	100			79	21		93	7		100			100		
	E	20	45	40	15	100			100			45	55		20	45	35		15	85	90		10	100			65		35	100			100		
Enterococcus faecium	C	14							100									100						100						100					
	E	17							100									100						100						100					
Enterococcus spp.	C	28							100									100						100						100					
	E	25							100									100						100						68		8			
Klebsiella oxytoca	C	19	67	11	22	78	11	11	89	11		89	11		89	11		34	22	44	100			100			78	22		100			89	11	
	E	18	89		11	100			100			34	33	33				72		28	67	22	11	100			22		78	100			100		
Klebsiella spp.	C	32	57	39	4	61	37	2	70	30		63	37		70	30		28	61	11	87	13		78	22		63	37		98	2		76	24	
	E	28	94	6		100			100			33	28	39				94		6	44	28	28	94		6	17		83	100			100		
Pseudomonas spp.	C	47				100			86	5	9	81	19		86	14					86	14					86	14					95	5	
	E	30				100			100			73	27		77		23				70	10	20				87		13				100		
S. aureus	C	33	87	13		87	13		83	17		87	4	9				17		83				87	13					100			96		5
	E	27	100			100			35		65	73	19	8				35		65				46		54				38		62	100		

*C: clinical, E: Environmental

AMC: Amoxycillin/Clavulanic acid, TZP: Piperacillin/Tazobactam, LVX: Levofloxacin, CRO: Ceftriaxone, FEP: Cefepime, AMP: Ampicillin, AMK: Amikacin,

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