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Multidrug-resistant bacteria isolated from patients hospitalized in Intensive Care Unit in University Hospital of Constantine, Algeria (2011 - 2015)

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The incidence of infections caused by multidrug resistant bacteria is increasing worldwide. The frequent misuse of antibiotic drug has greatly contributed to worldwide dissemination of antibiotics resistance. Multi-drug resistance in Gram-negative and Gram-positive bacteria causes a wide range of infections, particularly in the Intensive Care Unit (ICU) settings leading to an increased impact on morbidity, mortality and costs. This study was undertaken to determine the prevalence of multidrug resistant of bacterial isolates in patients admitted in ICU of university hospital of Constantine. We analyzed a 5-year period, from 2011 to 2015. Over five years period, 7472 clinical samples were collected in the Clinical Microbiology Laboratory of Benbadis University Hospital in Constantine. Identification of the isolates was performed by API automated systems (bioMérieux, Marcy l'Etoile, France) and automate microscan walkaway 96 (Siemens). Antibiotic resistance was determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion test on Mueller-Hinton Agar. Multidrug-resistant isolates included in this study were methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, Enterobactericeae that produce extended-spectrum beta lactamases and/or carbapenemases, multidrug-resistant *Acinetobacter baumannii* and multidrug-resistant *Pseudomonas aeruginosa*. A total of 3528 isolates were collected from various specimens such as blood (47.05%).The *Staphylococcus sp*, *Klebsiella sp*, *Acinetobacter sp*, *P. aeruginosa* and *E. coli* are the most common isolates recovered from clinical specimens in ICU (26.3, 18.7, 14.3, 11.9 and 9.2% respectively). MRSA strains constituted over 65% of all *S. aureus* isolates and 30.3% of *E. faecium* were found to be vancomycin resistant. Extended spectrum β-lactmase producers were expressed in 53.2% and 50.6 from *K. pneumoniae* and *E. coli*. Carbapenem resistance among *K. pneumoniae* improved slightly from 2.89 to 4.21%. *A. baumannii* isolates exhibited extremely high levels of resistance to all antibiotics except colistin (100% sensitive). In addition, 80.4% of *A. baumannii* isolates were found to be resistant to imipenem. Imipenem resistant *P. aeruginosa* isolates showed 36.4%.

Key words: Multi-drug resistant bacteria, Intensive care unit, Gram negative bacteria, Gram positive bacteria.

INTRODUCTION

The increase and spread of multidrug resistant (MDR bacteria have become a major concern worldwide. The hospital acquired infections caused by MDR infections caused by MDR bacteria have led not only to

an increase in mortality, morbidity, and cost of treatment, but also continue to endanger the life of patients (Martin and Yost, 2011; Delle Rose et al., 2015).

MDR bacteria can cause a wide range of infections, including bacteremia, pneumonia, urinary tract infection, peritonitis etc., which can lead to substantial morbidity and mortality, particularly in the ICU settings (Chen et al., 2016).

The risk of acquiring MDR bacteria in the ICU is increased by severity of illness, length of stay, use of intravascular devices, exposure of ICU patients to invasive therapeutic procedures like endotracheal intubation, the intensity of exposure to infected patients and the frequent misuse of antibiotic drug (Khan et al., 2014; Royer et al. 2015; Wroblewska et al., 2006).

A review of emerging MDR bacterial pathogens in ICU includes methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE) and multi-drug resistant Gram-negatives (Nayak et al., 2014; Thompson and FRCP, 2004; Russotto et al., 2015; Trubiano and Padiglione, 2015).

Infection and colonization with MRSA may be more frequent in the ICU than in general wards (Fridkin and Gaynes, 1999) and its resistance is conferred by the acquisition of one of several staphylococcal cassette chromosome mec elements that carry a gene (mecA) that encodes a penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics (Katayama et al., 2000). Vancomycin-resistant enterococci (VRE) have emerged as a nosocomial pathogen especially in ICUs worldwide (Austin et al., 1999). VRE are among the major health care-associated MDR organisms causing a serious problem in choosing an appropriate therapy (Schouten et al., 2008).

Infections caused by Gram-negative bacteria have features that are of particular concern. Specifically, the rate of infections related to MDR gram-negative bacteria (MDR-GNB) in ICU is increasing. These organisms are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic drug resistance, especially in the presence of antibiotic selection pressure (Russotto et al., 2015).

The emergence of resistance in Enterobacteriaceae is considered an alarming health threat. During this last decade, a growing number of *K. pneumonia*e and *E. coli* have developed resistance against third-generation cephalosporin, due to extended-spectrum β-lactamases (ESBLs) (Pitout and Laupland, 2008). Carbapenems are the first line treatment of ESBLs producing bacteria, and the emergence of carbapenem-resistant isolates conferred by New Delhi Metallo-β-lactamase-1 (NDM-1) leaves limited therapeutic options.

Non - fermentative Gram-negative bacilli (NF-GNB) mainly *A. baumannii* and *P. aeruginosa*, have emerged worldwide and the resistance of these organisms to antibiotics, particularly to carbapenems, has posed important therapeutic challenges. Currently, carbapenems are considered the antimicrobials of choice for treatment of serious infections caused by *A. baumannii* and *P. aeruginosa* but their efficacy is increasingly compromised by resistance as reported worldwide (Al Jarousha et al., 2009). This resistance has been attributed to the production of carbapenem-hydrolysing-lactamase enzymes of Ambler molecular class D (oxacillinases) and B (metallo- lactamases) (Woodford et al., 2011).

Despite its importance, a few studies on the MDR bacteria isolated from patients hospitalized in ICU were investigated in Algeria.

The aim of the present study was to determine the prevalence of MDR Gram positive and Gram negative organisms isolated from patients hospitalized in ICU of a university hospital in Constantine, Algeria.

MATERIALS AND METHODS

Isolation site

This study was undertaken in the Laboratory of Clinical Microbiology at the University Hospital of Constantine, Algeria, over five years period 2011- 2015.

Clinical samples for microbiological culture comprised blood, urine, pus, body fluid or aspirates (joint fluid, pleural fluid, ascitic fluid and cérébrospinal fluid), other diverse material (such as catheter, sonde and swab) and others like sputum. Isolates were collected from 7472 patients suspected of bacterial infection from a single ICU patients. Only one isolate per patient infection episode was included in this study.

Microbiological cultures and identification

All samples collected were aseptically inoculated on the various media and incubated at 37°C for 24 h. Cultures were processed using standard microbiological methods. Identification of the isolates was performed by API automated systems (bioMérieux, Marcy l'Etoile, France) and confirmed on the basis of the results of automate Microscan Walkaway 96 (Siemens).

Susceptibility testing

Antibiotic resistance was determined by the Clinical and Laboratory Standards Institute (CLSI) disk diffusion test on Mueller-Hinton Agar, using calibrated inoculum of the isolates based on McFarland standard, with the following antibiotics according to the bacterial species: piperacillin, trimethoprim—sulfamethoxazole, cefotaxime, gentamicin, amikacin, ceftazidime, ciprofloxacin, pefloxacin, imipenem, amoxicillin/clavulanic acid, ticarcillin, cefazolin, colistin and vancomycin. The results were interpreted according to the

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Table 1. Number and rate of positive culture from all samples.

Year	Total of samples	Positive isolates	Contamined isolates	Negative isolates
2011	1641	765 (46.61)	86 (5.24)	790 (48.14)
2012	1711	799 (46.69)	83 (4.85)	829 (50.89)
2013	1629	751 (46.10)	78 (4.78)	800 (49.10)
2014	1367	696 (50.91)	85 (6.21)	586 (42.86)
2015	1124	517 (45.99)	94 (8.36)	513 (45.64)
Total	7472	3528 (47.21)	426 (5.70)	3518 (47.08)

Table 2. Nature of the analyzed samples.

Sample	2011	2012	2013	2014	2015	Total (%)
Blood	319	326	384	388	243	1660 (47.05)
Tracheal	133	183	138	100	36	590 (16.72)
Urine	91	112	76	138	97	514 (14.56)
Sonde	118	81	83	10	77	369 (10.45)
Catheter	57	32	31	24	36	180 (5.10)
Pus	22	42	19	14	15	112 (3.17)
Body fluid	16	16	20	21	10	83 (2.35)
Others	9	7	0	1	3	20 (0.56)
Total	765	799	751	696	517	3528 (100)

guidelines of Clinical and Laboratory Standards Institute 2007 (CLSI). The antimicrobial susceptibility results for the six most frequently isolated organisms were analyzed in the present study. MDR isolates included in the analyses were MRSA, vancomycin-resistant enterococci (VRE), Enterobactericeae (*K. pneumoniae* and *E. coli*) that produce extended-spectrum beta lactamases and/or carbapenemases (ESBLs and CREs, respectively), Imipenem- resistant *A. baumannii* and *P. aeruginosa*. ESBL-producing strains of Gram-negative rods were detected with a double disk method using amoxicillin/clavulanic acid, ceftazidime, cefotaxime and ceftriaxone disks.

Resistance of NFGNB to imipenem was verified by Etest (AB Biodisk). MIC of vancomycin was also determined by using E-test method for enterococci.

RESULTS

Among the 7472 samples collected, 3528 were positive (47.21% of total samples), 426 were contaminated and represented 5.70% of total samples and 47.08% of all samples were negative (Table 1).

The bacterial isolates were obtained from 8 different specimens with the following percentage representations: blood (47.05%), tracheal (16.72%), urine (14.56%), sonde (10.45%), catheter (5.10%), pus (3.17 %), different body fluids (2.35%), and others (0.56%) (Table 2). In which, 2398 (67.97%) were Gram negative bacteria and 1130 (32.02%) were Gram positive (Figure 1). The commonest organism isolated from all samples was *Staphylococci* sp. 931 (26.38), *Klebsiella* sp. 661 (18.73) followed by *Acinetobacter* sp. 506 (14.34), *Pseudomonas* sp. 422 (11.96), *E. coli* 326 (9.24), *Enterobacter* sp. 227 (6.43) and *Enterococcus* sp. 117 (3.31). Others organisms were isolated but in small proportions: *Proteus* sp. 83 (2.34), *Streptococcus* sp. 82 (2.32), *Serratia* sp. 81 (2.29), *Shigella* sp. 32 (0.9), *Providencia* sp. 24 (0.68), *Haemophylus influenzae* 15 (0.42), *Morganella* sp. 9 (0.25), *Stenotrophomonas* sp. 6 (0.17), *Salmonella* sp. 4 (0.11) *and Citrobacter* sp. 2 (0.06) (Table 3).

Among Gram-positive isolates, strains of *Staphylococcus* sp. amounted to 82.38% (931/1130) and strains of *Staphylococcus aureus* constituted 37.8% (352/931). Whilst isolates of coagulase-negative staphylococci (CNS) constituted 62.19% (579/931). Of all strains of *Staphylococcus sp*, 80.98% (754/931) were methicillin-resistant. The proportion of methicillin-resistant increased from 70.68 in 2011 to 87.33 in 2014.

MRSA comprised 65.9% of all *S. aureus* isolates (232/352) and methicillin-resistant CNS (MRCNS) constituted 90.15% (522/579). The proportion of MRSA decreased from 76.54% in 2012 to 66.66% in 2015. 82.14% of CNS were methicillin resistant in 2011 and the rate of methicillin resistance raised 93.65% in 2014. In MRSA, there was no resistance to vancomycin.

Among Gram-positive bacterial strains, *enterococci* comprised 10.35% (117/1130). Out of 117 isolates, 84 (71.79%) were *Enterococcus faecalis* and 33 (28.20%) were *Enterococcus faecium*. No other enterococcal species were isolated during the study period.

No vancomycin-resistant *Enterococci* were observed during the first two years of our study, 2 isolates (14.28%), 6 (21.42) and 2 (11.11) of *enterococci* were found to be resistant to vancomycine, in 2013, 2014 and 2015 respectively. Ten out of 33 isolates of *E. faecium* (30.3%) were resistant to vancomycin , 2 strains (40%) in 2013, 6 strains (75%) in 2014 and 2 strains (28.57%) in 2015. However, no strains of vancomycin-resistant *E.faecalis* were detected in the study (Table 4).

Klebsiella sp. ranked second overall among ICU patients (18.73%) and was the most frequently isolated Gram-negative organism (27.56%). *K. pneumoniae* was most common specie 608/661 (91.98). For *K. pneumoniae*, the highest resistance rate was for ticarcillin, cefazolin and amoxicillin/clavulinat (100, 85.29 and 79.82%, respectively). All strains of *K.*

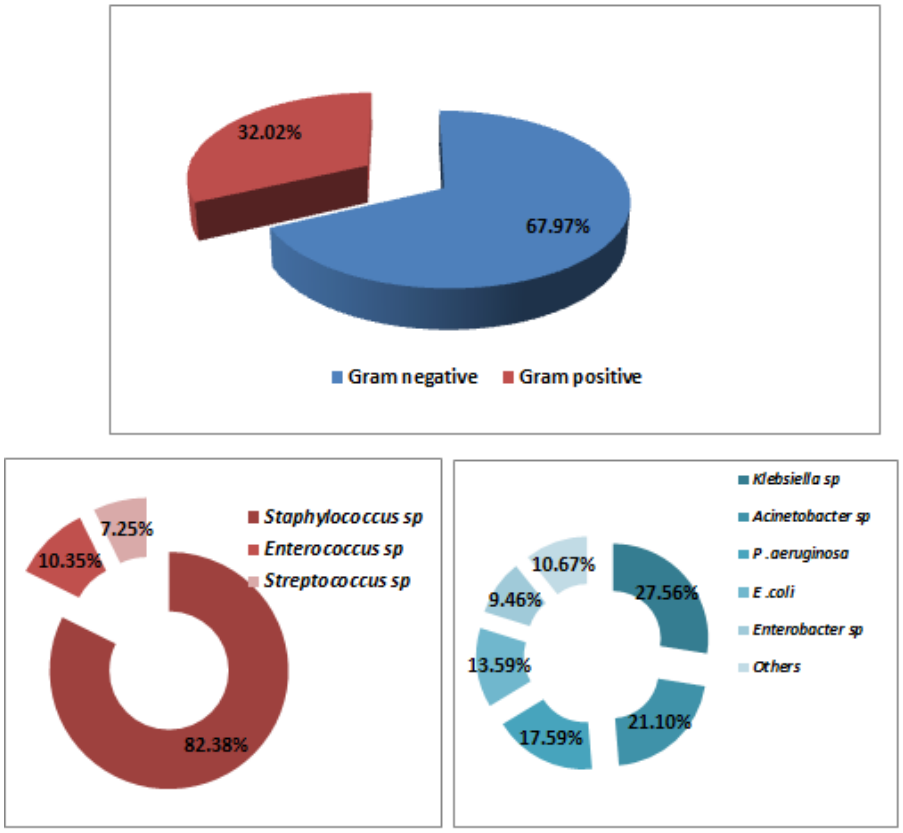

Figure 1. Prevalence of Gram positive and Gram negative isolates.

Table 3. Prevalence of clinical isolates.

Organisms	2011	2012	2013	2014	2015	Total(%)
Staphylococcus sp.	174	218	203	150	186	931(26.38)
Klebsiella sp.	124	143	154	140	100	661(18.73)
Acinetobacter sp.	112	96	119	107	72	506(14.34)
Pseudomonas aeruginosa	138	110	80	64	30	422(11.96)
Escherichia coli	60	65	70	84	47	326(9.24)
Enterobacter sp.	61	61	47	38	20	227(6.43)
Enterococcus sp.	31	26	14	28	18	117(3.31)
Proteus sp.	30	13	17	12	11	83(2.34)
Streptococcus sp.	18	22	12	14	16	82(2.32)
Serratia sp.	12	23	22	15	9	81(2.29)
Shigella sp.	0	0	0	30	2	32(0.90)
Providencia sp.	0	11	5	6	2	24(0.68)
Haemophilus influenzae	4	8	3	0	0	15(0.42)
Morganella sp.	0	1	4	3	1	9(0.25)
Stenotrophomonas sp.	0	0	0	5	1	6(0.17)
Salmonella sp.	0	1	1	0	2	4(0.11)
Citrobacter sp.	1	1	0	0	0	2(0.05)

pneumoniae were sensitive to colistin (Table 5).

ESBL production was detected in 53.28% of strains of *K.pneumoniae*. The proportion of ESBL increased from 52.06% in 2011 to 63.15% in 2015 (Table 6). Carbapenem resistance among *K. pneumoniae* improved slightly in 2013, 2014 and 2015 from 2.89 to 4.21%. *E.*

Table 4. Prevalence of multidrug resistant staphylococci and enterococci stratified by year.

Sample	Resistant isolate no./Total isolate no. (%)/ year					
	2011	2012	2013	2014	2015	Mean
Methicillin-resistant *Staphylococcus* sp.	123/174(70.68)	179/218(82.11)	172/203(84.72)	131/150(87.33)	149/186 (80.1)	754/931(80.98)
Methicillin-resistant *Staphylococcus aureus*	100/146 (68.49)	62/81 (76.54)	29/59 (49.15)	13/24 (54.16)	28/42 (66.66)	232/352 (65.9)
Methicillin-resistant *Staphylococcus* coagulase negative	23/28 (82.14)	117/137 (85.4)	143/144 (99.3)	118/126 (93.65)	121/144 (84)	522/579 (90.15)
Vancomycin resistant enterococci	0/31 (0)	0/26 (0)	2/14 (14.28)	6/28 (21.42)	2/18 (11.11)	10/117 (8.54)
VR *E. faecalis*	0/25 (0)	0/19 (0)	0/9 (0)	0/20 (0)	0/11 (0)	0/ 84 (0)
VR *E. faecium*	0/6 (0)	0/7 (0)	2/5 (40)	6/8 (75)	2/7 (28.57)	10/33 (30.3)

coli isolates from ICU patients exhibited elevated extended-spectrum β-lactamase (ESBL)-phenotype rates (50.61%) but the proportion of ESBL decreased from 61.66% in 2011 to 42.55% in 2015. the highest resistance rate for *E.coli* was for ticarcillin (82.65%), amoxicillin/clavulinat (64.57%) and cefazolin (63.11%). No strains showed resistance to imipenem and colistin.

NFGNB comprised 26.3% (928/3528) of all isolates. Among Gram negative isolates, strains of *Acinetobacter* sp. and *P.aeruginosa* comprised 21.1% (506/2398) and 17.59% (422/2398) of isolates respectively. Among strains of *Acinetobacter sp*, 93.87% (475/506) were *A.baumanii*.

In the present study, imipenem resistant was detected in 80.42% of *A. baumanii*, and 36.84% of *P. aeruginosa*. We recorded an increase of imipenem-resistant strains of *A. baumannii* during the study period from 60.39% in 2011 to 94.2% in 2014. The proportion of imipenem resitance increased slightly from 33.33% in 2011 to 50% in 2015 for *P.aeruginosa* (Table 6).

A. baumanii showed very high resistance rates to most antimicrobial agents. The highest resistance rates for *A.baumanii* were for piperacillin (97.54%), ticarcillin (97.52%) and ceftazidime (95.82%), while *P. aeruginosa* exhibited high susceptibility to most antimicrobial agents tested and the highest resistance rates was for pefloxacin (49.8%), piperacillin (37.82%) and ceftazidime (30.80%). All NFGNB isolates were sensitive to colistin.

DISCUSSION

Most isolates were recovered from the blood specimens (47.05%). This finding corroborated the results reported by other investigator in India (Jitendra et al., 2012). While in the ICU of Kingdom of Saudi Arabia (KSA), most isolates were recovered from the respiratory specimens (38.8%), followd by the blood specimens (33%) (Saeed et al., 2010).

Staphylococci constituted the group of bacteria most commonly isolated from ICU patients (Johnson et al., 2003). The commonest organism isolated from all samples was *Staphylococci* (26.38%) in our study. The most common GP cocci in this study were SCN, followed by *S.aureus*, whereas in the study of Fridkin et al. (1999), the most common GP cocci were *S. aureus*, followed by SCN.

In the present study, MRSA strains constituted over 65% of all *S. aureus* isolates. MRCNS strains represented 90.15% of CNS strains. These are high percentages from MRSA and MRCNS compared with data from other reports (Khan et al., 2014; Wang et al., 2011).

MRSA, first described in the 1960, is now endemic in many hospitals, infection and colonization with MRSA may be more frequent in the ICUs than in general wards (Thompson and FRCP, 2004).

Antibiotic susceptibility profile of MRSA showed that these strains exhibited the highest sensitivity to vancomycin (100%) In our study, *Enterococci* are important pathogens of patients hospitalized in the ICU, particularly in view of the increasing frequency of resistance to vancomycin. In this study, strains of *enterococci* comprised 3.31% of all bacterial isolates and 10.35% of Gram-positive isolates, this observation agreed with the finding of Johnson et al. (2003). Among *enterococci* isolates, 71.79% were *E. faecalis* and 25.46% were *E. faecium*. In the study of Sood et al. (2008), *E. faecalis* has been the predominant enterococcal species accounting for 80-85% of clinical isolates, followed by *E. faecium* which accounts for about 10-15% of clinical isolates.

In the present study, 10 (8.54%) isolates were found to be vancomycin resistant, 10 out of 33 isolates (30.3%) of *E. faecium* but no strains of vancomycin-resistant *E. faecalis* were detected. It has also been found in various studies that *E. faecium* accounts for far fewer clinical enterococcal isolates than *E. faecalis*, but it is far more resistant to glycopeptides.

In a study conducted by Saeed et al. (2010), less than 7.1% of *E. faecalis* were found to be resistant to vancomycin, whereas 40.1% of the *E. faecium* isolates were resistant to vancomycin. Zhanel et al. (2003) found that most of the VRE were *E. faecium* (88%), while only 12% were of *E. faecalis*. The Gram-negative organisms most frequently isolated in our study were *Klebsiella*

Table 5. Antimicrobial resistance of major four isolates Gram negative, stratified by year.

Organism/antimicrobial agent	Resistance rate (%) / Year					
	2011	2012	2013	2014	2015	Mean
Acinetobacter baumanii	**n=101**	**n=95**	**n=108**	**n=102**	**n=69**	
Ticarcillin	100	95.78	98.14	98.03	95.65	97.52
Piperacillin	98.01	97.89	98.14	98.03	95.65	97.54
Ceftazidime	95.04	98.89	94.44	95.09	95.65	95.82
Amikacin	72.27	57.89	70.37	66.66	86.95	70.82
Gentamycin	73.26	92.63	84.25	91.17	95.65	87.39
Ciprofloxacin	85.14	87.36	88.88	98.03	81.15	88.11
Pefloxacin	89.10	91.57	92.59	91.17	81.15	89.11
Trimethoprim-sulfamethoxazole	93.06	89.47	92.59	96.07	95.65	93.36
Colistin	0	0	0	0	0	0
Pseudomonas aeruginosa	**n=138**	**n=110**	**n=80**	**n=64**	**n=30**	
Ticarcillin	28.98	36.84	25	26.56	36.66	30.80
Piperacillin	16	24.6	27.5	84.37	36.66	37.82
Ceftazidime	23.91	11.81	18.75	78.12	20	30.51
Amikacin	4.34	3	3.75	79.68	23.33	22.82
Gentamycin	18	27.1	22.5	64.06	20	30.33
Ciprofloxacin	8	25.4	15	50	50	29.68
Pefloxacin	30	44	60	65	50	49.8
Trimethoprim-sulfamethoxazole	72.46	94.54	90	90.62	83.33	86.19
Colistin	0	0	0	0	0	0
Klebsiella pneumoniae	**n=121**	**n=125**	**n=138**	**n=129**	**n=95**	
Ticarcillin	100	100	100	100	100	100
Amoxicillin/clvulinat	66.94	88	86.95	78.29	78.94	79.82
Cefazolin	84.29	86.4	90.57	88.37	76.84	85.29
Imipenem	0	0	2.89	4.65	4.21	2.35
Amikacin	23.14	6.4	25.36	29.45	21.05	21.08
Gentamycin	74.38	81.6	61.59	66.66	54.73	67.79
Ciprofloxacin	41.32	38.4	21.73	48.06	33.68	36.63
Trimethoprim-sulfamethoxazole	67.5	82.64	74.63	68.21	56.84	69.96
Colistin	0	0	0	0	0	0
Escherichia coli	**n=60**	**n=65**	**n=70**	**n=84**	**n=47**	
Ticarcillin	95	70.76	94.28	75	78.72	82.65
Amoxicillin/clavulinat	41.66	92.30	68.51	52.38	68.08	64.57
Cefazolin	46.66	100	50	67.85	51.06	63.11
Imipenem	0	0	0	0	0	0
Amikacin	6.66	13.84	2.85	44.04	8.51	15.18
Gentamycin	38.33	66.15	18.57	21.42	34.04	35.70
Ciprofloxacin	43.33	21.53	80	47.61	46.8	47.85
Trimethoprim-sulfamethoxazole	66.66	62.5	52.30	55.95	53.19	58.12
Colistin	0	0	0	0	0	0

sp. (27.56%), *Acinetobacter* sp. (21.1%), *P. aeruginosa* (17.59%), and *E. coli* (13.59%). Similarly, these pathogens were the most frequently isolated Gram-negative organisms from ICU patients in Lybia (Zorgani et al., 2015). Whilst, the most common Gram-negative organisms in order of frequency from ICU patients were *Klebsiella* sp., *E. coli*, and *P. aeruginosa* in the USA and *E. coli*, *P. aeruginosa* and *Klebsiella* sp. In Europe (Sader

Table 6. Prevalence of multidrug resistant of major four isolates Gram negative, stratified by year.

Isolate	Resistant isolate no./Total isolate no. (%)/ Year					
	2011	2012	2013	2014	2015	Mean
ESBL-producing *K. pneumoniae*	63/121 (52.06)	57/125(45.6)	62/138 (44.92)	82/129 (63.56)	60/95 (63.15)	324/608 (53.28)
Carbapenemase-producing *K. pneumonia*	0/121 (0)	0/125 (0)	4/138 (2.89)	6/129 (4.65)	4/95 (4.21)	14/608 (2.3)
ESBL-producing *E. coli*	37/60 (61.66)	38/65(58.46)	30/70 (42.85)	40/84 (47.61)	20 /47 (42.55)	165/326 (50.61)
Imipenem- resistant *A.baumannii*	61/101 (60.39)	73/95 (76.84)	90/108 (83.33)	93/102 (91.17)	65/69 (94.2)	382/475 (80.42)
Imipenem–resistant *P. aeruginosa*	46/138 (33.33)	35/110 (31.18)	30/80 (37.5)	25/64 (40.62)	32/64 (50)	168/456 (36.84)

et al., 2014).

Over the past decade there has been a dramatic increase in MDR-Gram-negative rods, particularly among isolates recovered from ICU patients. In our study, ESBL production was detected in 53.28 and 50.61% of strains of *K. pneumoniae* and *E. coli* respectively. These data are similar to the proportion of 56.2 and 43.6% of ESBL-producing isolates amongst *E. coli* and *K. pneumoniae* isolated in clinical samples in China (Wang et al., 2008) but it is far higher than the same proportions reported in France (8 and 13%, respectively) (Arnaud et al., 2013). Whereas it is a less percentage compared with others reports in the literature. An Algerian's report of the resistance of bacteria to antibiotics (2012) have reported 76.73% of ESBL-producing isolates amongst *K. pneumoniae* in 2012. Saeed et al. (2010) found that ESBL producing *E. coli* were approximately 100% among the tested isolates, which were more common than that seen in *K. pneumoniae* (92%).

Carbapenem-resistant *K. pneumoniae* has emerged during recent years in several intensive care unit (Debby et al., 2012). Carbapenem resistance among *K. pneumoniae* improved slightly in 2013 and 2015 from 2.89% to 4.21% in the present study. Increasing rates of carbapenem resistance among Enterobacteriaceae have been reported by other investigators in various European countries (Mouloudi et al., 2010; Nordmann et al., 2011; Walsh, 2010). Rates of 2.3% and 38% of carbapenem resistance among *K. pneumoniae* have been reported (Sękowska et al., 2014; Chaudhary and Payasi (2013). KPC-producing strains appear to have the widest distribution, but a rising number of OXA-48–producing strains has been reported (Glasner et al., 2013).

In African countries, KPC producers were first identified by Brink et al. in 2012, in South Africa. In Algeria, a two recent studies described the first report of KPC-3-producing *K. pneumonia* (Bakour et al., 2015) and First outbreak of OXA-48-positive carbapenem-resistant *K. pneumoniae* isolates in ICU (Cuzon et al., 2015)**.** Strains of *P. aeruginosa* constituted 11.96% of all isolates ranking fourth in this study. *P. aeruginosa* was commonly isolated in ICU patients, ranking first in KSA (21.41%), second in Europe (18.2%) and China (15.6%), third in the USA (17.2%) and lybia (20%) (Saeed et al., 2010; Zorgani et al., 2015; Sader et al., 2014; Tan et al., 2014). It constituted 17.59% of Gram-negative strains in our study. This data is almost similar to the 24% reported from ICUs in Europe (Hanberger et al., 1999).

In the present study, the percentage of imipenem-resistant strains of *P. aeruginosa* was 36.84%. The proportion of imipenem resitance increased from 33.33% in 2011 to 50% in 2015. This is high compared with reports in the literature ranging from 2 to 20% (Glupczynski et al., 2001; Hsueh et al., 2001).

Strains of *Acinetobacter* sp. constituted 14.34% of all isolates and 21.10% of GN organisms. This is a high percentage compared with data from USA ICUs (Sader et al., 2014), but it is similar to others reports (Saeed et al., 2010; Tan et al., 2014). In present study, *A. baumanii* showed very high resistance rates to most antimicrobial agents and an important decrease in the susceptibility was observed for imipenem during the five years study period (60.39% in 2011 to 94.2% in 2015). This is very high compared with reports in the literature ranging from 9.6% to 23.8% (Wroblewska et al., 2006). While, among the four hundred and fifty four isolates of *A.baumani* isolated in India's ICU, 81.71% isolates were found to be carbapenemase producing (Chaudhary and Payasi, 2013).

The global spread of carbapenem-resistant *P. aeruginosa* and *A. baumannii* is of great concern (Hanberger et al., 1999). The significantly low susceptibility of ICU-acquired strains to carbapenems may be related to the increasing use of carbapenems in ICU. The most active compound against these organisms was colistin.

Conclusion

This study showed that *Staphylococcus sp*, *Klebsiella sp*, *Acinetobacter sp*, *P. aeruginosa* and *E. coli* were the most common isolates recovered from clinical specimens in the ICU of Constantine. Blood specimens represented 47.06% of all specimens collected in the ICU. This study demonstrated that most of these pathogens isolated from clinical samples were MDR. We conclude that the incidence of high rates of resistance is alarming high and is continuously increasing and spreading. Therefore surveillance of bacterial prevalence and susceptibility

patterns of the isolates from ICU is crucial in determining optimum empirical therapy of infections in critically ill patients.

Conflict of Interests

The authors have not declared any conflict of interests.

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