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Molecular Characterization of Resistance Genes in MDR-ESKAPE Pathogens

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In the last decade, along with the problem of nosocomial infections, multidrug-resistant bacteria in community and hospitals have soared. High frequencies of multidrug-resistant bacteria have been grouped under the acronym ESKAPE which capable of 'escaping' the biocidal action of antimicrobial agents. The objective of this study is to consider molecular characterization of resistance genes in ESKAPE pathogens. In this study, three hundred and eighty four bacteria were isolated from clinical samples in Loghman-Hakim Hospital, Tehran, Iran. MDR strains reported through disk diffusion method based on CLSI guidline. Molecular characterization of resistance genes were examined by PCR method. The prevalence of MDR-strains of *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter Spp.* were 3(9.09%), 17(23.3%), 11(11.22%), 18(18.36%), 9(18.91%), 4(5.8%), respectively. The most prevalence of resistant genes in ESKAPE bacteria were following as:*van*A 40.90%,*van*B 22.72% in *Enterococcus faecium*;*mec*A24.65%,*van*A4.11%, *van*B1.37%in *Staphylococcus aureus*;bla_{TEM} 28.57,bla_{KPC} 12.24% in *Klebsiella pneumonia*;bla_{SHV} 56.75%,bla_{VIM} 32.43%,bla_{TEM} 29.73 %in *Acinetobacter baumannii*; bla_{SHV} 44.70%,bla_{OXA} 55.29%,bla_{VEB} 74.11%,bla_{VIM} 62.35%,bla_{PER} 62.35%,Mex-A 74.11% ,Mex-B 81.17%, Mex-R76.47% , bla_{PER} 62.35% in *Pseudomonas aeruginosa*; bla_{TEM} 39.13%, bla_{SHV} 33.33% in *Enterobacter* SPP. Knowledge of resistance genes prevalence in ESKAPE pathogens is necessary to prepare feasible data about tracing and treatment of infection related to these microorganisms that may be beneficial to clinicians to select a convenient empirical therapeutic diet in diseases due to ESKAPE pathogens at the bedhead. It is recommended that healthcare-associated, community-acquired, and nosocomial infections to be clearly considered annually.

Keywords: Molecular characterization, Resistance genes, ESKAPE pathogens.

Importance of ESKAPE in clinics

The ESKAPE pathogens able to get away from antibiotics biocidal action and generally show new example in pathogenesis, transmission trace and resistance pattern. The ESKAPE group contains *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter spp*[1]

Recently, understanding of virulence, resistance, transmission and pathogenicity of these microbes cause to innovative strategies for the progress of new antimicrobial options. Directing attention towards ESKAPE will help to concentrate antimicrobial resistance (AMR)

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challenge and allow efficient critical assessment of new antimicrobial drugs. Unfortunately, increasing resistance to treatment in ESKAPE has been recognized in recent years[2]

***Enterococcus faecium* (E)**

One of the parts of normal intestinal flora of most humans is Enterococcus genus[3, 4] The two most significant species, *Enterococcus faecalis* and *Enterococcus faecium*, cause various human infections such as septicemia, bacteremia, urinary tract infections (UTI), endocarditis, neonatal sepsis meningitis, and wound infections[5, 6] Some resistant species e.g. High-level aminoglycoside-resistant (HLAR) enterococci and vancomycin-resistant enterococci (VRE) have been emerged and cause great difficulties in treatment[7-9] There are nine vancomycin resistance genes contain van A, B, C, D, E, G, L, M, and N. The most predominant types in worldwide are *van*A and *van*B[10-12]. Gene *van*A, relates to a high degree resistance to teicoplanin and vancomycin, is mostly conferred to vancomycin resistant *Enterococcus faecium*[13]. Gene *van*B relates to a vancomycin high level resistant but susceptibility to other glycopeptides such as teicoplanin whereas previous antibiotics able to induce the *van*B resistance type[14].

***Staphylococcus aureus* (S)**

A common agent of the skin microbiota and generally is isolated from moist anatomical areas is *S. aureus*. Nearly 60% of the human population harbor *S. aureus* irregularly as intermittent carriers; whereas around 20% of individuals almost always carry a single *S. aureus* strain as persistent carriers. Even, *S. aureus* is a common wound pathogen, cause both acute and chronic infections by biofilm formation.

After the first clinical reports of methicillin resistance *S.aureus* (MRSA) in 1960s, via *mec*A expression which encodes a low affinity penicillin-binding protein (PBP2a), MRSA has expended resistance against b-lactam antibiotics including all b- lactams such as penicillins, cephalosporins, and carbapenems. First-line drug of choice for infections due to MRSA is mostly glycopeptide antibiotics such as vancomycin or teicoplanin .Unfortunately, being intensive selective pressure has caused to emerge vancomycin-intermediate *S. aureus* (VISA) ,and vancomycin-resistant *S. aureus* (VRSA)[2]

***Klebsiella pneumonia* (K)**

Being common isolated strain in health care setting, emergence of MDR *K. pneumoniae* and spread easily makes this microorganism as a main nosocomial pathogen to cause infections such as bacteremia, septicemia and urinary tract infections (UTIs) in children .Typically, MDR *K. pneumoniae* have been resistant to several different classes of antibiotics such as aminoglycosides, quinolones , b-lactams, and b-lactamase inhibitors. With the passage of time by means of the generation of their new mutant strains, resistant to antimicrobials drugs in MDR *K. pneumoniae* will become more and more[15]

As a results, carbapenem-resistant Enterobacteriaceae especially carbapenem-resistant *K. pneumoniae* (CRKP),are increasingly implicated in sporadic worldwide outbreaks due to multiple combinations of extended-spectrum b-lactamases (ESBLs) and carbapenemases by means of the dissemination of mobile genetic platforms related to encoding every class of b-lactamase.(2)

***Acinetobacter baumannii* (A)**

A great challenge for physicians and clinical microbiologists is management of MDR *Acinetobacter* spp. infections. Capability to survive in a health care setting makes it a common agent for healthcare-associated infections and lead to multiple outbreaks. (16-18) spectrums of infections due to MDR *Acinetobacter* spp. contain bacteremia, meningitis, pneumonia, UTI, and wound infection[19-24]

A. baumannii is intrinsically resistant to antibiotics constitutively due to express active efflux pump systems, the low-quantity expression of small-aperture outer membrane porins; possess a resistance island, which includes a cluster of genes encoding antibiotic and heavy metal resistance which impart resistance to ammonium-based disinfectants. The broad acquisition of ESBLs in some isolates confers with resistance to all known antimicrobials, containing imipenem and colistin.This combination of intrinsic virulence and multiple resistance factors, makes *A. baumannii* as symbol the superbug .So current clinical demand for novel antimicrobials is necessary[25, 26]

***Pseudomonas aeruginosa* (P)**

There are some differences in the medical society to the definition of MDR, so the

real incidence of MDR *P. aeruginosa* is not well proved[27] Most of the time, MDR was described as resistance to at least three antimicrobial drugs from a different classes of antibiotics, mostly penicillins, cephalosporins, aminoglycosides, carbapenems, antipseudomonal, and fluoroquinolones. Annually, different geographic places and centers limit the ability to determine the right percentage of MDR *P. aeruginosa* spread[28,29] Current articles have emphasized that above mentioned agents may or may not be as impressive as first-line drugs, but may as well as be pertaining to more considerable adverse effects (i.e. ototoxicity ,nephrotoxicity ,and neurotoxicity)[30-38]

Antibiotic therapy may induce expression or select for stably depressed mutants, resulting in resistance to ticarcillin, piperacillin and third-generation cephalosporins. Most common resistant mechanisms are usually due to the metallo-b-lactamases or MBLs (such as IMP, VIM, SPM and GIM), combination of low outer membrane permeability and multidrug efflux systems, overexpression of nodulation-cell division (RND) family of transporters (e.g., MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM), detriment of the outer membrane protein (porin) OprD, and other mechanisms such as enzyme production and target mutations. Expression of acetyltransferases, nucleotidyl transferases and phosphotransferases (enzymes related to aminoglycoside-modifying and aminoglycoside resistance), are common too[38-65].

***Enterobacter* Species (E)**

Enterobacter spp., most commonly cause the urinary and respiratory tracts, bloodstream and serious nosocomial infections, displaying broad MDR via plasmid-encoded ESBLs and carbapenemases, such as KPC, verona integron-encoded metallo-b-lactamase, OXA and even metallo-²-lactamase-[66,67]. In addition to colistin and tigecycline, few antimicrobials drugs are effective against these resistant organisms .There are little or no drugs in the 'pipeline' that are known to be capable of effectively addressing this mounting health crisis[2, 68]

In veterinary medicine, fluoroquinolone resistance is become to increase. This resistance is occurred by both chromosomal and plasmid-mediated fluoroquinolone resistance (PMQR) mechanisms which accompany with other antimicrobial resistance genes containing b-lactamases. The genes' relationship with PMQR can cause resistance to fluoroquinolone when joined with topoisomerase mutations and efflux pumps[69, 70]

So surveillance studies about ESKAPE can help governments to provide a public health plan to decrease use of improper antibiotics in infections caused by ESKAPE. In the last decade, along with the problem associated with nosocomial infections, MDR bacteria in community and hospitals have exceed. The objective of this study is to molecular approaches to resistance genes in ESKAPE pathogens.

MATERIALS AND METHODS

Sample collection

In this descriptive study, 384 bacteria were isolated from clinical samples (such as trachea, urine, wound, discharges, ascites fluid, pleural fluid, blood, synovial fluid, and catheter) in Loghman-Hakim Hospital, Tehran, Iran.

Identification ESKAPE pathogens

Laboratory identification of *Enterococci faecium* was done by Gram staining, growth on blood agar, hydrolysis of esculin with blackening of bile esculin agar, negative catalase production, and growth on 6.5% sodium chloride[71-73]

The isolates were identified as *Staphylococcus aureus* based on morphologic and biochemical tests such as: Gram stain, catalase, coagulase, hot-cold ²-hemolysin on blood agar, DNAase and mannitol salt agar fermentation[74] All the strains were screened for methicillin resistance by means of oxacillin (1 µg) and cefoxitin (30 µg) examination by disk diffusion test method, based on the standard guidelines[75]

For identification of *Klebsiella pneumonia* and *Enterobacter Spp.*, the samples were cultured on nutrient agar, MacConkey agar, blood agar and eosin methylene blue (EMB) agar (All of media were purchased from Hi Media Company, India). After incubation of plates at 35°C for 24 h , the pure isolates identified based on Gram stain and biochemical tests such as; catalase, oxidase, indole production, citrate utilization, sugar metabolism reaction on triple sugar iron agar, urea test, orthonitrophenyl-²-galactoside (ONPG) test, and methyl red Voges-Proskauer(MRVP), as described

in standard bacteriological methods. All of the above chemicals and media were purchased from Sigma-Aldrich, Germany[76, 77]

For identification of *Acinetobacter baumannii*, we used standard bacteriologic and biochemical methods, which contained Gram staining, catalase tests, oxidase tests, motility, oxidation/fermentation (O/F) tests, citrate utilization tests, and capability to grow at 37 and 44° C[78]

Pseudomonas aeruginosa is a non-fermenting Gram negative rod which often related to human infection. It is a strict aerobe with a growth temperature range of 5-42°C and colonies with characteristic grape-like smell of aminoacetophenone. The blue-green appearance of pus or of an organism culture is pertaining to the combination of pyocyanin (blue pigment) and pyoverdin (fluorescein, yellow pigment). Other pigments such as pyorubin (red) or pyomelanin (brown) was produced by several strains. Almost all strains are motile by means of a single polar flagellum. At least six colonial types were produced by *P. aeruginosa* on nutrient agar after 24hr aerobic incubation at 37°C. Colonies isolated on selective or blood agar identified by a positive oxidase reaction, Gelatinase positive reaction and characteristic pigment production as '*P. aeruginosa*'[79]

Resistant Gene detection in ESKAPE pathogens

By AccuPrep Genomic DNA extraction kit (cat.no.k-3032 lot no.1008J, BIONEER) DNA was extracted from all GBS isolates. PCR amplification profile comprised a 300 nM concentration of each oligonucleotide primer (Eurofins MWG Operon); 200 mM (each) deoxynucleoside triphosphates dCTP, dGTP, dATP, and dUTP; 0.125 U of Taq DNA polymerase; and 5.5 mM MgCl2 (from GENET BIO, prime Taq TM DNA polymerase,URL:www.genetbio.com)

The PCR products were analyzed by gel electrophoresis on 1.5% BIONEER agarose gels in 1X TBE buffer (890 mM of boric acid, 890 mMTris, 40ml of 0.5 M EDTA, pH 8.0) at 100 V for 60 min. Green loading buffer with DNA stain(Jena Bioscience ,Lot:111.034) was used during loading the samples and ladder. The sizes of the PCR products were determined by comparison with the molecular size standard (50bp-1Kb linear scale; low range DNA ladder or 100bp-3Kb linear scale and mid-range DNA ladder, Jena Bioscience. The rPSL gene was used for each reaction as housekeeping control gene primer sequences used in this study were presented in table 1.

RESULTS

In this study, 384 bacteria were isolated from clinical samples .We examined trachea 29 (7.5%), urine 74 (19.27%), wound 44 (11.45%), discharges 37(9.63%), ascites 26 (6.77%), pleural effusion 25 (6.5%), blood87 (22.66%), synovial fluid 31 (8.07%), and catheter 31 (8.07%) in Loghman-Hakim Hospital, Tehran, Iran. We isolated 22(5.72%) *Enterococcus faecium*, 73(19.01%) *Staphylococcus aureus*, 98(25.52%) *Klebsiella pneumonia*, 37(9.63%) *Acinetobacter baumanii*, 85(22.13%) *Pseudomonas aeroginosa*69 (17.96%) *Enterobacter* Spp.

According to CDC definition, MDRs are described as microorganisms that are resistant to one or more classes of antimicrobial drugs. The prevalence of MDR-strains of *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter Spp.* were 3(9.09%), 17(23.3%), 11(11.22%), 18(18.36%), 9(18.91%), 4(5.8%), respectively.

Antibiotic resistance pattern about *Enterococcus faecium* was ampicillin14(63.63%), penicillin17(77.27%), nitrofurantoin16(72.72%), tetracycline19(86.36%), linezolid1(4.54%), vanc omycin4(18.18%),gentamicin10(45.45%),strepto mycin5(22.72%),ciprofloxacin21(95.45%).

Staphylococcus aureus strains resistant pattern were following as:Erythromycin 69(94.52%), clindamycin57(78.08%), SXT 47(64.38%), vancomycin 2(2.74%), ciprofloxacin 71(97.26%), gentamicin 42(57.56%), minocycline 38(52.05%), rifampicin 51(69.86%), amikacin 64(87.67%), kanamycin 67(91.78%), oxacillin 36(49.31%), penicillin 72(98.63%). All of isolates were sensitive to linezolid.

Klebsiella pneumonia isolates were resistant to ampicillin 71(72.44%) ,cefazolin 84(85.71%) ,gentamicin 66(67.34%), tobramycin 22(22.44%), amikacin 39(39.79%), cefepime19(19.38%), ceftriaxone 21(21.42%),

ciprofloxacin 47(47.95%), imipenem 51(51.04%), meropenem 37(37.75%), piperacillin 87(88.77%).

Antibiotic resistant pattern against to *Acinetobacter baumannii* were ceftazidime34 (91.89%), ciprofloxacin33(89.19%), imipenem28 (75.67%), meropenem32(86.48%), gentamycin27 (72.97%), tobramycin25(67.57%), amikacin36(97.29%), cefepime35(94.59%),ceftriaxon36(97.29%), tetracycline20(54.05%), piperacillin1(2.7%). All strains were resistant to SXT.

In evaluation of resistant pattern of *Pseudomonas aeruginosa* we detected gentamicin65 (76.47%), tobramycin14 (16.47%), amikacin65 (76.47%), cefepime13 (15.29%), ciprofloxacin66 (77.64%), imipenem63 (74.12%), meropenem69 (81.17%), piperacillin45 (52.94%), aztreonam 25 (29.41%), ceftazidim 39 (45.88%)

The resistant pattern of antimicrobial drugs about *Enterobacter* Spp. were ampicillin 68(98.55%), cefazolin 67(97.1%), gentamicin 5(7.25%), tobramycin 4(5.79%), amikacin 2(2.89%), cefepime 43(62.32%), ceftriaxone 39(56.52%), ciprofloxacin 12(17.39%), imipenem 1(1.45%), meropenem1 (1.45%), piperacillin3 (4.35%).

Based on table 1, the most prevalence of resistant genes in ESKAPE bacteria were following as: *van*A 40.90%,*van*B 22.72% in *Enterococcus faecium*;*mec*A24.65%,*van*A4.11%,*van*B1.37%in *Staphylococcus aureus*;bla_{TEM}28.57,bla_{KPC} 12.24% in *Klebsiella pneumonia*;bla_{SHV} 56.75%,bla_{VIM} 32.43%,bla_{TEM} 29.73 %in *Acinetobacter baumannii*; bla_{SHV} 44.70%,bla_{OXA} 55.29%,bla_{VEB} 74.11%,bla_{VIM} 62.35%,bla_{PER} 62.35%,Mex-A 74.11% ,Mex-B 81.17%, Mex-R76.47% , bla_{PER} 62.35% in *Pseudomonas aeruginosa*; bla_{TEM} 39.13%, bla_{SHV} 33.33% in *Enterobacter* SPP.

Table 1. Primer sequences and PCR condition were presented in this study

Genes	Oligonucleotide sequences (52 to 32)	Size (Bp)	Ref.
bla_{TEM}	GGGACARTCSKATGAATGTCA GGGYSGCTTAGATAGTGCTG AT	425	80
bla_{SHV}	GGTTATGCGTTATATTCGCC TTAGCGTTGCCAGTGCTC	867	81
bla_{OXA}	ACACAATACATATCAACTTCGC AGTGTGTTTAGAATGGTGATC	885	82
bla_{CTX-M}	ATGTGCAGYACCAGTAARGT TGGGTRAARTARGTSACCAGA	593	81
bla_{DHA}	CACACGGAAGGTTAATTCTGA CGGTTATACGGCTGAACCTG	970	83
bla_{VIM}	CTTCATTCACGCACTATTAC TAACTTGACCGACAGAGG	827	84
bla_{PER}	ATTCGTATGCTGGATCTCGCCACC CATGACCCAGTTCGCCATATCCTG	396	84
bla_{FOXUP}	AGTGGTGAGTATCCGACAG ATGAAAGTGCGTGGAGAC	225	84
bla_{GES}	GGTCAAGGATCTGGATTTGG ACATGCGTGTAAATCATCGTC	500	84
$bla_{NHAmp\ C}$	CACCACGAGAATAACCGCCTTGAACTCGACCG	1184	84
bla_{VEB}	CGACTTCCATTTCCCGATGC GGACTCTGCAACAAATACGC	1014	85
bla_{SPM-1}	GTACAAGGGATTCGGCATCG TGGCCTGTTCCCATGTGAG	569	86
bla_{KPC}	TCTGGACCGCTGGGAGCTGGTGCCCGTTGACGCCCAATCC		87
bla_{IMP}	GGAATAGAGTGGCTTAAYTCTCCCA AACYACTASGTTATCT	188	88
Mex-A	CTCGACCCGATCTACGTC GTCTTCACCTCGACACCC	503	89
Mex-B	TGTCGAAGTTTTTCATTGAG AAGGTCAC GGTGATGGT	280	89
Mex-R	GAACTACCCCGTGAA TC CACTGGTCGAGGAGATGC	411	89
OprM	GATCCCCGACTACCAGCGCCCCG ATGCGGTACTGCGCCCGGAAGGC	247	89
OprD	ATCTACCGCACAAACGATGAG GCCGAAGCCGATATAATCAAACG	156	89
rPsL	GCAAGCGCATGGTCGACAAGA CGCTGTGCTCTTGCAGGTTGTGA	201	89
*mec*A	TCCAGATTACAACTTCACCAGG CCACTTCATATCTTGTAACG	162	90
*van*A	CATGAATAGAATAAAAGTTGCAATACCCCTTTAACGCTAATACGATCAA	1030	91
vanB	GTGACAAACCGGAGGCGAGGA CGCCATCCTCCTGCAAAAAA	433	82
vanC	ATCCAAGCTATTGACCCGCTTGTGGCAGGATCGTTTTCAT	402	93
*van*C-2/3	ATCCAAGCTATTGACCCGCTGTAGGAGCACTGCGGAACAA	582	93

Statistics: Data were analyzed using IBM TM SPSS 20 software.

DISCUSSION

Health crisis of ESKAPE pathogens seems overwhelming. It is necessary that the last remaining antimicrobial agents be protected against intellectual choice and ameliorated infection control. Selection of suitable guidelines is readily accessible and accurate prescribing protocols have been successfully implemented worldwide[2] Briefly, healthcare-associated, community-acquired, and nosocomial infections should be carefully considered. Knowledge of residential antimicrobial resistance can protect the selection of a convenient empirical therapeutic regimen in which diseases due to ESKAPE pathogens[94]

For appropriate therapy, knowing the antibiotic resistance pattern in ESKAPE pathogens is essential, therefore we examined the distribution of antibiotic resistance in mentioned microorganisms. To our knowledge, this is the first assessment of the ESKAPE pathogens in Iran. There were different results about the resistant pattern of antibiotics in ESKAPE pathogens.

The prevalence of MDR-strains of ESKAPE pathogens were following as: *Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter Spp.* were 3(9.09%), 17(23.3%), 11(11.22%), 18(18.36%), 9(18.91%), 4(5.8%), respectively.

We reported that antibiotic resistance pattern about *Enterococcus faecium* was ampicillin14(63.63%), penicillin17(77.27%), nitrofurantoin16(72.72%), tetracycline19(86.36%), linezolid1(4.54%) ,vancomycin4(18.18%), gentamicin10(45.45%) , streptomycin 5(22.72%), ciprofloxacin 21(95.45%). Increasing antibiotic resistance in common bacterial pathogens, in both hospitals and communities, present a growing threat to human health worldwide. VRE

Table 2. The prevalence of resistance genes related to multiple drug resistance in ESKAPE pathogens

Genes	*E. faecium* N (%)	*S.aureus* N (%)	*K.pneumonia* N (%)	*A.baumanii* N (%)	*P.aeroginosa* N (%)	*EnterobacterSpp.* N (%)
bla_{TEM}	NT	NT	28(28.57)	11(29.73)	12(14.11)	27(39.13)
bla_{SHV}	NT	NT	11(11.22)	21(56.75)	38(44.70)	23(33.33)
bla_{OXA}	NT	NT	5(5.10)	2(5.40)	47(55.29)	12(17.39)
bla_{CTX-M}	NT	NT	6(6.12)	1(2.70)	5(5.88)	12(17.39)
bla_{DHA}	NT	NT	6(6.12)	1(2.70)	13(15.29)	8(11.59)
bla_{VEB}	NT	NT	4(4.08)	1(2.70)	63(74.11)	3(4.35)
bla_{GES}	NT	NT	7(7.14)	0	24(28.23)	2(2.9)
bla_{VIM}	NT	NT	8(8.16)	12(32.43)	53(62.35)	2(2.9)
bla_{FOXUP}	NT	NT	6(6.12)	1(2.70)	11(12.94)	7(10.14)
$bla_{NHAmp\ C}$	NT	NT	5(5.10)	1(2.70)	5(5.88)	7(10.14)
bla_{PER}	NT	NT	5(5.10)	1(2.70)	53(62.35)	3(4.35)
bla_{IMP}	NT	NT	4(4.08)	0	14(16.47)	3(4.35)
bla_{SPM}	NT	NT	1(1.02)	0	0	2(2.9)
bla_{KPC}	NT	NT	12(12.24)	NT	5(5.88)	7(10.14)
Mex-A	NT	NT	NT	NT	63(74.11)	NT
Mex-B	NT	NT	NT	NT	69(81.17)	NT
Mex-R	NT	NT	NT	NT	65(76.47)	NT
OprM	NT	NT	NT	NT	19(22.35)	NT
OprD	NT	NT	NT	NT	25(29.41)	NT
rPsL	NT	NT	NT	NT		NT
*mec*A	NT	18(24.65)	NT	NT	NT	NT
*van*A	9(40.90)	3(4.11)	NT	NT	NT	NT
*van*B	5(22.72)	1(1.37)	NT	NT	NT	NT
*van*C	1(4.54)	0	NT	NT	NT	NT
vanC-2/3	1(4.54)	0	NT	NT	NT	NT

NT: not tested

is an important subject in health care setting. Accompany with increased spread , their ability to transfer resistance genes related to vancomycin to other bacteria (such as MRSA) have bring up them as a subject of discussion and intense study. In our study, the Enterococci were most resistant to several antibiotics that were different with the other studies[72,95-99].

As above mentioned, *Staphylococcus aureus* strains resistant pattern were following as*:* Erythromycin 69(94.52%), clindamycin 57 (78.08%), SXT 47(64.38%), vancomycin 2(2.74%), ciprofloxacin 71(97.26%), gentamicin 42(57.56%), minocycline 38(52.05%), rifampicin 51(69.86%), amikacin 64(87.67%), kanamycin 67(91.78%), oxacillin 36(49.31%), penicillin 72(98.63%). All of isolates were sensitive to linezolid.

The worldwide emergence of MRSA is a remarkable challenge for public health based on centers for disease control (CDC) reports, 1% of all Staphylococcal infections and 50% of healthcare-associated Staphylococcal infections are caused by MRSA[74]

There is different prevalence of MRSA in the world. Twelve percent of MRSA strains were detected in 2015 in PIRC Tehran, Iran[74], 11.3% in Germany, in 2007, (100) and 17.57% west of Iran, in 2013 (101). Compared to studies in Germany (6.5%), The Netherlands (1.4%), Shiraz, Iran (5.3%), Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran (3.2%), Switzerland (3.3%), the USA (3.4%), France (6.6%) and the UK (6.7%), the prevalence of MRSA strains were lower than in our study (102-109). Rezaei et al. considered colonization of MRSA and MSSA in atopic dermatitis patients. They found a higher rate (33%) of MRSA colonization in the nasal cavity. The MRSA was one of the most frequent organisms that were found on their skin[110]. The MRSA isolates showed variable resistance to clindamycin, ceftriaxone, cefpodoxime, azithromycine, and erythromycin[111]. Resistance to penicillin and clindamycin[111, 112] was similar with the other studies.

By definition, all MRSA isolates can take the *mec*A gene, which allows resistance to all ²-lactam drugs, containg cephalosporins and carbapenems. In our study and in similar studies, several MRSA are susceptible to a number of beta lactams, such as cephalosporins[113-115].

Klebsiella pneumonia isolates were resistant to ampicillin 71(72.44%), cefazolin 84(85.71%), gentamicin 66(67.34%), tobramycin 22(22.44%), amikacin 39(39.79%), cefepime19(19.38%), ceftriaxone 21(21.42%), ciprofloxacin 47(47.95%), imipenem 51(51.04%), meropenem 37(37.75%), piperacillin 87(88.77%).

The other of own studies in 2012, in consideration of 19 ESBL producing K. *pneumonia*, the frequency of gene groups were detected as following: bla_{CTX} (94.73%), bla_{TEM} (94.73%), and *blaSHV* (78.94%)[77]

In Gholipour A and et al study, ESBL genes were detected in 18.75% of *E. coli* and *K. pneumonia* isolates. They identified the bla_{TEM} gene was detected in 12.14% of *E. coli*, in the event that it was not diagnosed in *K. pneumonia*. The bla_{SHV} gene was identified in 7.47% of *E. coli* and 14.28% of *K. pneumonia* isolates. The lowest rates of resistance were detected for: tazocin (6.25%), amikacin (12.5%) and gentamicin (14.84%). Resistance to other ones were as follows: "nitrofurantoin (16.4%), nalidixic acid (23.43), co-trimoxazole (25%), cefepime (32%), ciprofloxacin (55.46%), ceftazidime (59.76%), ampicillin (69.53%) and cefotaxime (73.43%)"[116]

According to Abujnah AA and et al study in 2015, they reported that *Klebsiella* Spp. were 100% resistant to ampicillin, 33.3% to ceftriaxone, and 17.4% to ciprofloxacin. Forty two percent of *Klebsiella* spp. isolates were MDR.Totally; bla_{TEM} gene was identified in 7 isolates, bla_{OXA} gene in 10 isolates and $bla_{CTX\text{-}M}$ gene in 6 isolates. We could not recognized bla_{SHV} gene in the present study[117]

Antibiotic resistant pattern against to *Acinetobacter baumannii* were *recognized* ceftazidime34 (91.89%),ciprofloxacin33(89.19%),imipenem28(75.67%),meropenem32(86.48%),gentamycin27(72.97%),tobramycin25(67.57%),amikacin36(97.29%),cefepime35(94.59%),ceftriaxon36(97.29%),tetracycline20(54.05%),piperacillin1(2.7%).All strains were resistant to SXT.

According to Safaria M and et al study, they recognized 87 (87%), 95 (95%), 98 (98%) and 95 (95%) out of 100 *A. baumannii* isolates were resistant to imipenem, meropenem, ceftazidime and cefotaxime, respectively. Also, phenotypically, 99% and 7% of the isolates were MBLs and ESBLs

produced. Out of 100 *A. baumannii* isolates, 13 (30%) harbor the bla_{VIM}-family and 20 (20%) and 58 (58%) have been confirmed to carry TEM and SHV genes, respectively[118]

In evaluation of resistant pattern of *Pseudomonas aeruginosa* were detected that gentamicin65 (76.47%), tobramycin14 (16.47%), amikacin65 (76.47%), cefepime13 (15.29%), ciprofloxacin66 (77.64%), imipenem63 (74.12%), meropenem69 (81.17%), piperacillin45 (52.94%), aztreonam 25 (29.41%), ceftazidim 39 (45.88%). Thus, studies in large-scale surveillance have keep in sight the alteration in *P. aeruginosa* susceptibilities pattern over time but have not figured out the underlying mechanisms responsible for the enhancement in *P. aeruginosa* resistance[119-122]

The resistant pattern of antimicrobial drugs about *Enterobacter* Spp. were ampicillin 68(98.55%), cefazolin 67(97.1%), gentamicin 5(7.25%), tobramycin 4(5.79%), amikacin 2(2.89%), cefepime 43(62.32%), ceftriaxone 39(56.52%), ciprofloxacin 12(17.39%), imipenem 1(1.45%), meropenem1 (1.45%), piperacillin3 (4.35%).

The ESBL propagation in Enterobacter spp. was approximately twofold as high as in compare with the ESBL outbreak in invasive E. coli and Klebsiella pneumonia isolates from the identical period in the Netherlands (4.7% and 6.9%, respectively).A presumably description for this diversity was the absence of a laboratory protocol for ESBL tracing in Enterobacter spp., eventuating in a lack of infection control measures and then an increased likelihood of nosocomial outbreak. Of the ESBL-producing strains, 40% were MDR, i.e., altogether resistant to ciprofloxacin, cotrimoxazole, and tobramycin or gentamicin, versus 3% in the non-ESBL isolates. With enhancing of MDR strains will increase the utilization of carbapenems; an unfavorable expansion in the face of the universal emergence of carbapenemase-producing Enterobacteriaceae strains[123,124].

It should be mentioned that the clinical response of a patient after receiving antibiotic does not always correlate with the laboratory reports. Even so, it should be noted that description of pathogens antimicrobial resistance patterns needs a consecutive update[125]

CONCLUSION

Similar to other developing countries, antimicrobial resistance pattern surveillance has not been awarded sufficient regard in our country. Outcomes of present study demonstrated that the rate of antibiotics resistance is growing in Iranian health care setting. So, Iranian health ministry should provide guideline protocol and appropriate programs for antibiotic therapy in hospitals particularly alongside the other physicians for prescribing suitable antibiotics for antibiotic resistance prevention, better remedy and evaluation of the patients soon. In addition, educational and medical systems in Iran need training some well-educated personnel on the prohibition and management of antibiotic resistance. Knowledge of resistance genes associated with ESKAPE pathogens is necessary to prepare useful data about tracing and treatment of infection related to these microorganisms and may be beneficial to clinicians for selection a convenient empirical therapeutic diet in diseases due to ESKAPE pathogens at the bed head. Surveillances of healthcare setting, community-acquired, and nosocomial infections is suggested annually. We must enhance hospital infection-control procedure for restricting resistance spread. This procedure will ensure a steady stream of new antibacterial drugs to meet the needs of current patients. Further research needed in this regards in Iran.

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