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(RESEARCH ARTICLE)

Correlation of multi-drug resistance integron genes, *blaESBL* carriage of Extended-spectrum β-lactamas producing clinical isolates from equine with respiratory disorders

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Abstract

The present study was aimed to identify phenotypical and genotypic antimicrobial characterization of clinical isolates obtained from equine with respiratory disorders in Egypt, as well as the correlation between the β-lactamases and integron genes in clinical isolates.

Thirty two clinical isolates were identified as *K.pneumoneae* (15 isolates); *P.aeruginosa* (8 isolates); *S.zooepidemicus* (3 isolates) and *S.equi* (6 isolates). All isolates showed resistance to more than 3 classes of antibiotic and were considered as multidrug resistance isolates (MRD isolates),

*K. pneum*oneae harbor ctx-M genes and shv-1 gene in highest rate (53.3% and 60% respectively). P.aeruginosa isolates harbor ctx-M genes and shv-1 gene (20% and 33.3% respectively) while S.equi and S.zooepidemicus harbor only ctx-M genes (33.3% and 20% respectively), tem-1 gene was observed in S. equi (66.7%) followed by K,pneumoneae (33.3%) and absent in P.aeruginosa. Significant correlation or association of intI genes with ctx-M genes in K. pneumoneae, P.aeruginosa and streptococcus species was observed, while it was significant with shv-1 in K.pneumoneae and streptococcus species. The significance was at $p<0.05$.

It was concluded that the detection of integrons in most of the bacterial isolates promoters the phenomenon of in depth resistance because of these mobile genetic elements therefore the detection of those mobile gene cassettes in equine may be a keystone of multidrug resistance. Companion animals predominantly contain bla-ctx-M clusters, which might be animal origin, causing quick changes in drug-resistance epidemiology. ctx-M-1 and shv-1 also are commonly found among human pathogens signifying a standard clonal heredity and a possible ESBL dissemination source in farm and livestock.

Keywords: Ctx-M genes; Shv-1 gene; Multidrug resistance MDR; Equine; ESBL β-lactamase

1. Introduction

Bacterial disease may consequence in mucosal bacterial infections (rhinitis and tracheitis) or may yield more serious invasive disease like pneumonia and pleuropneumonia. *S equi subsp. equi*, is that the cause of strangles. It's a main

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bacterial pathogen of the upper tract and is clever of mucosal invasion without predisposing factors. *Streptococcus equi subsp. zooepidemicus* is that the commonest compliant pathogen of the equine lung, although *K.pneumoneae* and *Pseudomonas aeruginosa* were frequently isolated. (Rush [1] and Nehal et. al [2]

The genus Klebsiella contains of non-motile, aerobic and facultative anaerobic, Gram negative rods (Abott , [3]). Quite 50 studies are realized during which molecular epidemiologic techniques are conducted to review outbreaks of infection with ESBL (Extended-spectrum beta-lactamases) producing Klebsiella spp. Paterson [3].

Resistance to β-lactams is especially because of the assembly of β-lactamase genes, which can be occur either on chromosomes or on plasmids (Bradford, [5]). Resistance to extended-spectrum β-lactams has been related to the presence of extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo-β-lactamases (MBLs) (Batchelor et al [6]).

Mobile genetic components like transposons and plasmids are described to hold genetic units named integrons which hold genes for site-specific recombination, and are adept of taking and assembling gene cassettes (Hall and Collis [7]; Stokes and Hall [8]).

The present study was pointed to spot phenotypical and genotypic antimicrobial characterization of clinical isolates obtained from equine with respiratory disorders in Egypt and study the correlation between the B-lactamases genes and integron genes in clinical isolates.

2. Material and methods

At Equine Bacterial Diseases in Animal Health Research Institute, 31 clinical isolates were obtained from 275 samples equine with respiratory disorders which characterized by mucopurlent nasal discharge, cough pneumonia with lung abscess (during the study period June 2019 until January 2021, samples underwent bacteriological examination wherever samples were cultured on buffer peptone water for enrichment and incubated at 37C for 18-24 hours, then cultured on agar , MacConkey agar (UK standard, [9]), and pseudomonas agar (CN media), UK standard [10]). All supposed colonies were further biochemical identified using S.R.O. GP24 and S.R.O. GN24 kits (diagnostics.S.R.O.TM), fifteen *Klebsiella pneumoneae*; 8 *P.aeruginosa* ; 3 *S.zooepiemicus* and 6 *S.equi* were subjected to susceptibility test. All isolates were kept in glycerol stocks at -20°C.

Antimicrobial susceptibility testing was done by agar disc-diffusion consistent with standards of the Clinical and Laboratory Standards Institute (CLSI [11])

The following antimicrobials were used: penicillin 10ug, oxacillin 1ug, ampicillin 10ug, ampicillin-sulbactam 10ug, ampicillin-clavulanic acid 30µg, piperacillin-tazobactam 110µg, cephalexin 30µg, cephradine 30µg, cefotaxime 30µg, cefoperazone 75µg, meropenem 10µg, aztreonam 300µg, clarithromycin10µg, erythromycin 15µg, oxytetracycline 30µg, chloramphenicol 30µg, norfloxacin 10µg, ofloxacin5µg, lomefloxacin 10µg, kanamycin 30µg, novobiocin 30µg, streptomycin10µg, neomycin10µg, amikacin 30µg, linezolid 30µg, amoxicillin- clavulanic acid 30µg, clindamycin 2µg, vancomycin 30µg and doxycycline 30µg (Oxoid).

2.1 Detection of resistance genes of MDR resistance genes

This study was designed to seek out different alleles of drug resistance genes (Table 1).

2.2 Extraction of DNA

Bacterial cells were grown on LB media and during a 1.5ml microcentrifuge tubes, it had been harvested (1-2ml of overnight bacterial culture (A600=1) may correspond to 1-2x109 cells), centrifuged for 1min at 12000rpm, supernatant was discarded and extraction of DNA was done according the manufacture guide of ABIOpure extraction kit. The concentration and quality of the extracted cellular DNA was assessed using spectrophotometer (Thermo Scientific) by estimating the optical density at a wave length of 260 and 280nm.

The concentration was calculated as follows:

1OD. 260 nm = 50 µg/ml
The purity of DNA = OD. At 260/OD.

Table 1 Target genes for antibiotic resistance genes by the 7-plex panel

Target gene		Alleles required
β-Lactamases		
1	blatem	including 1, 3, 10, 104–106, 71, 76–84, 138, 143, 150, 155
2	blashv	including 1, 2, 5, 11, 12, 25, 26, 38, 56
3	blactX-M group 1	including 1, 3, 15, 28, 29, 32, 36, 58, 79, 103
Integron genes		
4	intI	Integron class I
5	intII	Integron class II
6	intIII	Integron class III
Target genes for antibiotic resistance genes by the 2-plex panel		
1	AmpC blamox-M	CMY-1, CMY-8 to CMY-11, MOX-1, MOX-2
2	AmpC DHA-1	blaDHA beta lactamases from a range of enterobacteriaciae species

2.3 Molecular identification of *K.pneumoneae*

The reaction mixture for PCR amplification of 16S rRNA for *K. pneumoneae* consisting of 200ng of DNA template, 25µl master mix (MyTaq™ HS Red Mix) , 1µl of every primer was added and therefore the volume of mixture was completed by Dnase free water to 50µl. the amplification was done Table (2).

2.4 Multiplex PCR for β-lactamases genes and integron genes:

M-PCR for β-lactamases was applied using 200ng of DNA template with 1µl of every primer of β-lactamase genes, 25µl master mix (MyTaq™ HS Red Mix) and Dnase free sterile water was added to finish the quantity to 50µl. Extracted DNA was wont to find β-lactamase genes using gradient super cycler (kyratec). An equivalent technique was finished the three integron genes. The PCR programs and sequence of every primer were illustrated in Table (2).

2.5 Single plex for mox-M and DHA-1 genes

The PCR reaction in 25µl final volume includes: 12.5µl master mix (MyTaq™ HS Red Mix) 1µl of 10 pmol/ µl of primer), and sterile water was added to finish the quantity to 25µl. Extracted DNA was wont to detect mox-M and DHA-1 genes. The PCR program and sequence of primer were clarified in Table (3).

Table 2 The nucleotide sequences of the primers and type of PCR cycles used in this study

Target genes	Sequence	Size of amplification (bp)	cycle of PCR	References
KP_16S_NM3	F=5'-ATG TCG CAA GAC CAG AGT GG-3' R=5'-GCA CAA CCT CCA AAT CGA CA-3'	657	Initial denaturation:3min at 95 °C; 35cycles of: Denaturation: 20sec. at 95°C, Annealing :30sec. at 58°C, Extension: 30sec at 72°C; Final Extension :10min at 72°C Cooling or store: 10°C, at ∞	Aurna [13]

Phenotypic Mechanism for different primers of β-lactamases and integrons genes (using M-PCR)				
Pan- Tem	F=5'-CAG CGG TAA GAT CCT TGA GAG-3' R=5'-GAG TTA CAT GAT CC C CCA TG TT-3'	326	M-PCR Initial Denaturation: 5 min at 95 °C; 35cycles of: Denaturation: 20sec. at 95°C, Annealing :20sec. at 60°C, Extension: 1min at 72°C; Final Extension of 7min at 72°C Cooling or store: 4°C, at ∞	Schmidt *et al.* [40]
pan-SHV	F=5'-CGC CTG TGT ATT AT C TCC CTGT-3' R=5'-CAA GGT GTT TTT CGC TGA CC-3'	316		
CTX-M group 1	F=5'-GCA AAA ACT TGC CGA ATT AGA G-3' R=5'-GCT TAT TCA TCG CCA CGT TAT C-3'	320		
Integron genes (using M-PCR) Gradient thermocycler				
intI	F=5'-GCCTTGCTGTTCTTCTACGG -3' R=5'-GATGCCTGCTTGTTCTACGG -3'	558	M-PCR Denaturation: 5 min at 95 °C; 30 cycles of: Denaturation:1 min at 95°C, Annealing :1min at 65°C, Extension: 1min at 72°C; Final Extension of 10min at 72°C Cooling or store: 4°C, at ∞	Estabraghi, et al. [41] Ashayeri-Panah, et al. [43]
intII	F=5′-CACGGATATGCGACAAAAAGGT-3’ R=5′-GTAGCAAACGAGTGACGAAATG-3’	789		
intIII	F=5′-GCCTCCGGCAGCGACTTTCAG-3’ R=5′-ACGGATCTGCCAAACCTGACT-3’	979		
AmpC genes (single plex PCR)				
MOXM	F=5′-GCT GCT CAA GGA GCA CAG GAT-3’ R=5′-CAC ATT GAC ATA GGT GTG GTG C-3’	520	Single plex PCR Denaturation at: 94°C for 3 min. 30 cycles of: Denaturation:30s at 94°C Annealing :64°C for 30 s, Extension:1min at72°C Final extension of 7min. at 72°C Cooling or store: 10°C, at ∞ Cooling or store: 4°C, at ∞	Hoseini et al[44] Pérez-Pérez & Hanson [45]
DHA-1	F=5′-CTG ATG AAA AAA TCG TTA TC-3’ R=5′-ATT CCA GTG CAC TCA AAA TA-3’	1,136	Single plex PCR Denaturation at: 94°C for 3 min. 35 cycles of: Denaturation:1min at 94°C Annealing :55°C for 1min , Extension:at72°C for 1min Final extension for 7min. at 72°C Cooling or store: 4°C, at ∞	Yan, et al. [46]

Screening of PCR products by agarose gel electrophoresis (Sambrook, et al.[12])

One gram agarose was along with 100ml Tris acetate EDTA (TAE) buffer. The agarose was melted in microwave for 3 second and 0.4µl/100ml ethidium bromide and gone to chill at temperature. After hardening, the comb was detached and therefore the gel was applied to the electrophoresis cell. The cell was crammed with TAE buffer. 10µl of every of

the PCR product samples were applied to the gel together with 5µl relative molecular mass marker (MaestroGen DNA ladder 100-3000bp) The electrophoresis cell was covered and therefore the power supply was adjusted at 200volt for 30min. up to 1h. The gel was taken out from the cell and examined under radio wave UV transilluminator.

3. Results and discussion

All clinical isolates (31 isolates) obtained from samples of equine with respiratory disorders were bacteriologically identified as *K.pneumoneae subsp. pneumoneae* (15 *K.pneumoneae*); *P.aeruginosa* (8 isolates); *S.epui subsp. zooepidemicus* (3 *S.zooepidemicus*) and *S.equi.subsp.equi* (6 *S.equi*). On susceptibility antibiotic test, all isolates showed resistance to quite 3 classes of antibiotic and were considered as multidrug resistance isolates (MRD isolates).

All isolates of *K.pneumoneae* were molecular identified by KP_16S_NM3 primers at 657bp (Figure 1), this result agreed with Aurna, [13].

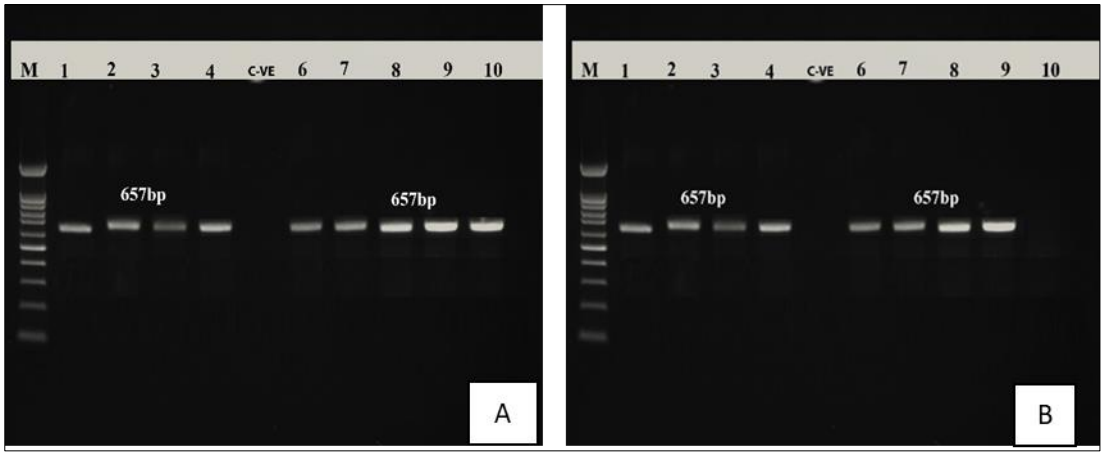


Figure 1 A: PCR products for 16S RNA of *K.pneumoneae*. Lane M:Ladder;lane 1: control positive; Lanes 2-4,6-10: positive samples for 16S RNA of *K.pneumoneae* Lane C-ve: control negative. B: Lane 1 Control positive *K,pneumpneae* ATCC 700721; 2-4, 6-9: positive samples for 16S RNA of *K.pneumoneae*

Table (3) and figure (2) revealed the phenotypic profile; numbers and severity of resistance of *K.pneumoneae* isolates to different antibiotics and antibiotic classes resistance, where 4 isolates showed the very best antibiotic resistance and antibiotic classes resistance (92 and 100% respectively). Two isolates showed resistance to 22 antibiotics antibiotic resistance (88%) and eight antibiotic classes resistance (88.8%), 4 isolates showed resistance to twenty antibiotics resistance and eight classes (80% and 88.8% respectively), but each two isolates has an equivalent profile. The remainder of isolates showed different intensity of antibiotics resistance and antibiotic class resistance.

Table 3 Phenotypic and genotypic profile of MRD isolates of *K.pneumoneae*

Number of isolates	Antibiotics	No. of resistant AB	% of resistant AB	Number of resistant AB classes	% of resistant AB classes	Genetic profile	Number of resistant AB genes	% resistant AB genes
1	P, AMP, S, AMC, TZP, CL, CTX, CEQ, CLR	9	6	5	55.5	ctx	1	6.7
2	P, AMP, AMC, TZP, CL, CTX, CEQ, C, ENR, NV, S, N	12	8	6	66.6	ctx	1	6.7
3	P, AMP, AMC, TZP, CL, CTX, CEQ, MEM,	16	64	8	88.8	ctx	1	6.7

	ATM, CLR, C, LOM, ENR, NV, S, N							
4	P, AMP, AMC, TZP, CL, CE, CFP CEQ, MEM, ATM, CLR, C, LOM, ENR, NV, N	16	64	8	88.8	shv,tem,intII	3	13.3
5	SAM, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, NV, S, N	18	72	8	88.8	shv	1	6.7
6	SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, ENR, NV, S, N	20	80	8	88.8	shv,int II	1	6.7
7	SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, ENR, NV, S, N	20	80	8	88.8	ctx,shv,intI	3	20
8	SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, ENR, NV, S, N	20	80	8	88.8	Shvs ,intII	2	13.3
9	SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, ENR, NV, S, N	20	80	8	88.8	shv	1	6.7
10	SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	22	88	8	88.8	ctx, intI	2	13.3
11	SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM,	22	88	8	88.8	ctx, intI	2	13.3

	ENR, NV, S, N, AK							
12	AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	23	92	9	100	shv, intI	2	13.3
13	AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	23	92	9	100	Shv, intI	2	13.3
14	AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	23	92	9	100	ctx,intI,intII	3	20
15	AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	23	92	9	100	ctx,mox, intI	3	20

P: Penicillin, OXA: Oxacillin, Amp: Ampicillin, SAM: Ampicillin-sulbactam, AMC: Ampicillin -clavulanic acid, PRL: Piperacillin-tazobactam, CFX: Cephalexin, CE: Cephradine, CTX: Cefotaxime, CPZ: Cefoperazone, CEQ: Cefquinome, MEM: Meropenem, ATM: Aztreonam, CLR: Clarithromycin, OXT: Oxytetracycline, C: Chloramphenicol, NOR: Norfloxacin, OFX: Ofloxacin, LOM: Lomefloxacin, ENR: Enrofloxacin, K: kanamycin, NO: Novobiocin, S: Streptomycin, N: Neomycin, AK: Amikacin, MDR: Multidrug resistant, PDR: Pan-drug resistant, n: Number, AB: Antibiotic

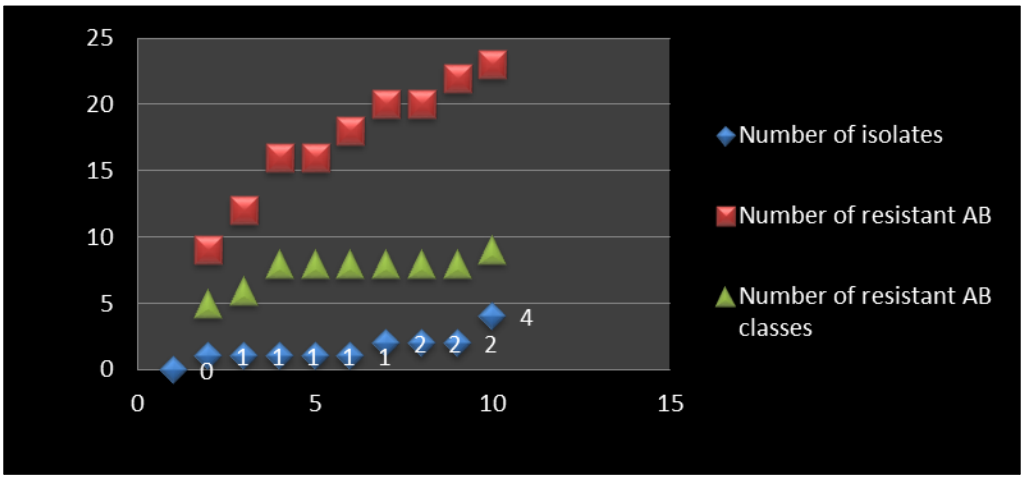


Figure 2 Distribution of antibitic resistance classes and antibiotic resisdstance among clinical isolates of *K. pneumoneae*

All *P. aeruginosa* isolates showed resistance to all or any antibiotic resistant classes (100%) but the phenotypic profile were differed, where 2 isolates had an equivalent profile to 23 antibiotic resistant, 2 isolates were just like 22 antibiotic resistance and three isolates were different in antibiotic resistance profile. (Table 4 & figure 3)

Table 4 Phenotypic and genotypic multidrug resistance drug (MDR) profile of *P. aeruginosa*

Number of isolates	Antibiotics	Number of resistant AB	%	Number of resistant AB classes	%	Genetic profile	Number of resistant AB genes	%
1	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, OFX, LOM, K, NV, S, N, AK	23	92	9	100	ctx,intIII	2	13.3
2	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, ENR, LOM, K, NV, S, N, AK	23	92	9	100	ctx, DHA-A,intI	3	20
3	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, CLR, OT, C, NO, OFX, LOM, K, NV, S, N, AK	23	92	9	100	shv.intII	2	13.3
4	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, OFX, K, NV, S, N, AK	22	88	9	100	shv	1	6.7
5	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, OFX, K, NV, S, N, AK	22	88	9	100	shv,intII	2	13.3
6	P, OXA, AMP, SAM, AMC, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, OFX, K, NV, S, N, AK	21	84	9	100	ctx,intII	1	6.7
7	P, OXA, AMP, SAM, AMC, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, LOM, K, NV, S, N, AK	21	84	9	100	shv	1	6.7
8	P, OXA, AMP, SAM, AMC, CL, CE, CTX, CFP, CEQ, MEM, CLR, OT, C, NO, OFX, ERN, NV, S, N, AK	21	84	9	100	shv	1	6.7

P: Penicillin, OXA: Oxacillin, Amp: Ampicillin, SAM: Ampicillin-sulbactam, AMC: Ampicillin -clavulanic acid, PRL: Piperacillin-tazobactam, CFX: Cephalexin, CE: Cephradine, CTX: Cefotaxime, CPZ: Cefoperazone, CEQ: Cefquinome, MEM: Meropenem, ATM: Aztreonam, CLR: Clarithromycin, OXT: Oxytetracycline, C: Chloramphenicol, NOR: Norfloxacin, OFX: Ofloxacin, LOM: Lomefloxacin, ENR: Enrofloxacin, K: kanamycin, NO: Novobiocin, S: Streptomycin, N: Neomycin, AK: Amikacin, MDR: Multidrug resistant, PDR: Pan-drug resistant, n: Number, AB: Antibiotic

Gram positive bacteria (9 clinical isolates) was tested for resistance phenotypic profile against 26 antibiotics representing 11 classes , *S.zooepidemicus* 2 isolates were just like 19 antibiotic resistance profile and therefore the third isolates showed resistance to 18 antibiotics but all isolates showed resistance to 9 (81.1%) antibiotic classes (Table 5 & chart 3). 4 clinical isolates of *S.equi* were identical in phenotypic resistance antibiotics (26 resistant antibiotics) and antibiotic resistant classes (!00%) and therefore the other 2 clinical isolates were different in their profile and also in antibiotic resistant classes (Table 5 and figure 5). This diversity of clinical isolates obtained from the tract matched the capacity of antibiotic microbial resistance (AMR) revealed the indiscriminate and extensive use of antibiotics which has led to the emergence and extent spread of resistant pathogenic bacteria (Wolska et al. [14]; Garza-Cervantes et al.[15])and are, therefore, reflected because the ESKAPE pathogens (Pendleton et al. [16]).

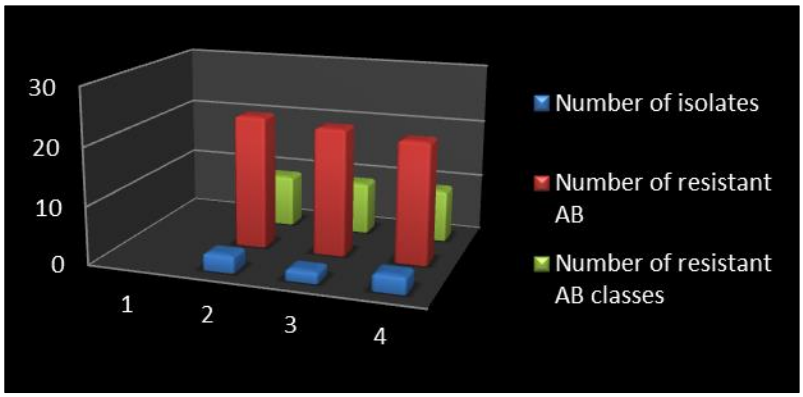


Figure 3 Distribution of antibiotic resistance and antibiotic classes resistance isolates of *P. aeruginosa*

Detection of ESBL and AmpC β-lactamases and integron genes were administered on AMR clinical isolates by using multiplex PCR and therefore the presence of those genes were recorded, where the very highest rate of ctx-*M* genes were observed in *K.pneumoneae* (53.3%) (Fig. 2) followed by *S.equi* (33.3%) Fig. (4), *P.aeruginosa* and *S.zooepidemicus* (20%) Fig. (3&9). Sulphydryl variable-type β-lactamases (shv-1 gene) also were detected in highest rate *in K.pneumoneae* (60%) (Fig. 6) followed by *P.aeruginosa* (33.3%) Fig. (7), and it had been absent in streptcoccus species. While the greatest rate of tem-1 gene was observed in *S.equi* (66.7%) Fig. (4), followed by *K.pneumoneae* (33.3%) (Fig. 6) and absent in both *P.aeruginosa* and *S.zooepidemicus* (Table 6 & fig..8). The genetic profile of the clinical isolates where recorded (Tables 3, 4, 5).

Table 5 Phenotypic and genotypic profile of *steptcoccus spp.*

Number of isolates	Antibiotics	Number of resistant AB	%	Number of resistant AB classes	%	Genetic profile	Number of resistant AB genes	%
S.equi Subsp. Zooepidemicus								
1	AMP, SAM, AMC, CE, CFP, CEQ, CLR, E, OTX, NOR, OFX, K, NV, S, AK, DA, VA	18	66.7	9	81.1	Ctx.intI	1	6.7
2	P, AMP, SAM, AMC, CE, CF, CEQ, CLR, E, OTX, NOR, OFX, LOM, K, NV, S, AK, DA, VA	19	70.4	9	81.1	ctx	1	6.7
3	P, AMP, SAM, AMC, CE, CF, CEQ, CLR, E, OTX, NOR, OFX, LOM, K, NV, S, AK, DA, VA	19	70.4	9	81.1	ctx	1	13.3
S.equi subsp. Equi								
3	P, OXA, AMP, SAM, AMC, CE, CTX, CFP, CEQ, MEM, CLR, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA, VA	26	100	11	100	ctx	1	13.3
4	P, OXA, AMP, SAM, AMC, CE, CTX, , CFP, CEQ, MEM, CLR, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA, VA	26	100	11	100	tem, intI	2	6.7

5	P, OXA, AMP, SAM, AMC, CE, CTX, CFP, CEQ, MEM, CLR, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA, VA	26	100	11	100	tem,intI	2	13.3
6	P, OXA, AMP, SAM, AMC, CE, CTX, CFP, CEQ, MEM, CLR, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA, VA	26	100	11	100	tem, intI	2	13.3

P: Penicillin, OXA: Oxacillin, Amp: Ampicillin, SAM: Ampicillin-sulbactam, AMC: Ampicillin-clavulanic acid, PRL: Piperacillin-tazobactam, CFX: Cephalexin, CE: Cephradine, CTX: Cefotaxime, CPZ: Cefoperazone, CEQ: Cefquinome, MEM: Meropenem, CLR: Clarithromycin, E: Erythromycin, OXT: Oxytetracycline, DO: Doxycycline, NOR: Norfloxacin, OFX: Ofloxacin, LOM: Lomefloxacin, K: kanamycin, NO: Novobiocin, S: Streptomycin, N: Neomycin, AK: Amikacin, LIN: Linezolid, DA: Clindamycin, VA: Vancomycin, MDR: Multidrug resistant, PDR: Pan-drug resistant, n: number. AB: Antibiotic

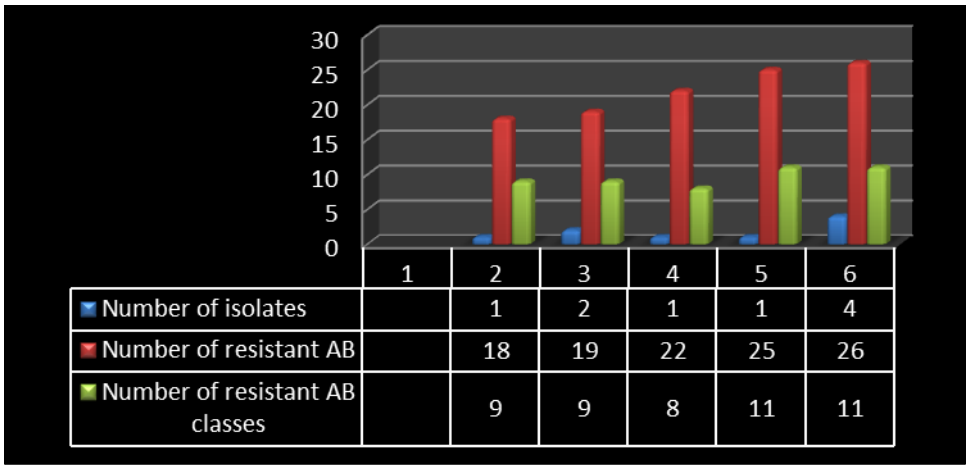


Figure 4 Distribution of antibiotic resistant and antibiotic classes resistant Streptcoccus species

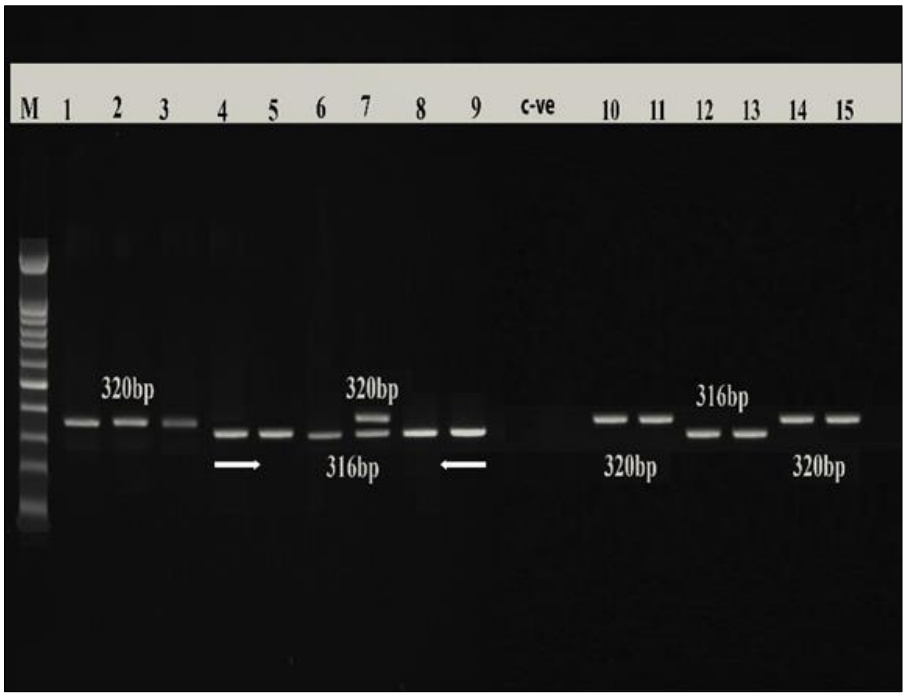


Figure 5 PCR products for B-lactamases genes in *K.pneumoneae*.Lane M: ladder; lanes 1;2;3 ctx-M lanes 4;5;6: shv-1. Lane7: ctx-M & shv-1. Lanes 8; 9; 10; 11; 14 & 15: ctx-M. Lane C-ve: control negative (water)

The family β-lactamases consists of the ctx-M enzymes, which are ESBLs, The designation *ctx-m* mirrors the hydrolytic activity of those β-lactamases against cefotaxime. they're allocated into five clusters on the idea of the aminoalkanoic acid sequence: ctx-M-1, ctx-M-2, ctx-M-8, ctx-M-9 and ctx-M-25 (Paterson & Bonomo[17]; Gupta[18])

More than 150 teM-type b-lactamases are detected, and every one of them are produced from tem-1 or tem-2 by mutations. In divergence to the common of tem β-lactamases, tem-1 isn't ESBLs and is merely capable to hydrolyse penicillins. Some TEM derivatives are proven to possess a reduced affinity for β-lactamases inhibitors and are termed inhibitor-resistant TEM. These enzymes have insignificant activity against extended-spectrum cephalosporins and aren't revealed to be ESBLs (Chaibi et. al [19]).

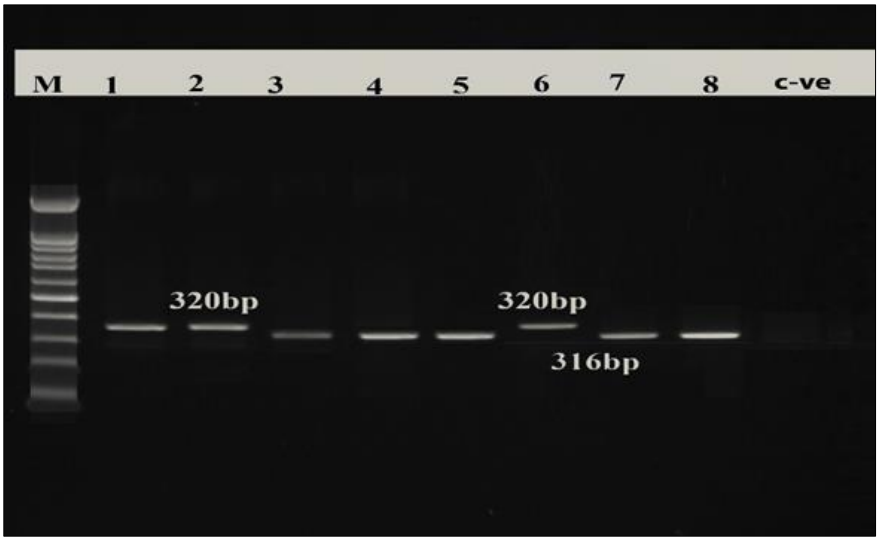


Figure 6 PCR products for B-lactamases genes in P.aeruginosa..Lane M: ladder; lanes 1;2ctx-M lanes 3;4;5 ;7&8:: shv-1. Lane 6: ctx-M & Lane C-ve: control negative (water)

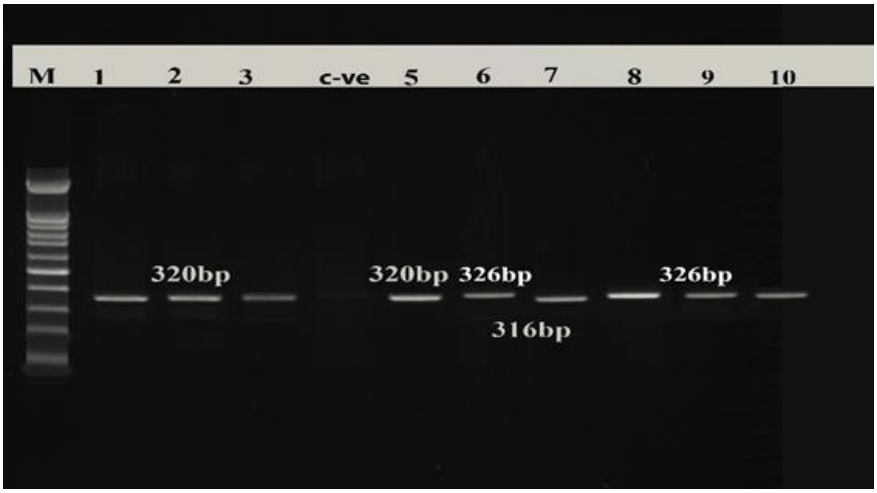


Figure 7 PCR products for B-lactamases genes in streptococcus species..Lane M: ladder; lanes 1;2 &: ctx-M in S.zooepidemicus. Lanes 5;7: ctx-M in S.equi. Lanes8:9 & 10 shv-1. C-ve: control negative (water)

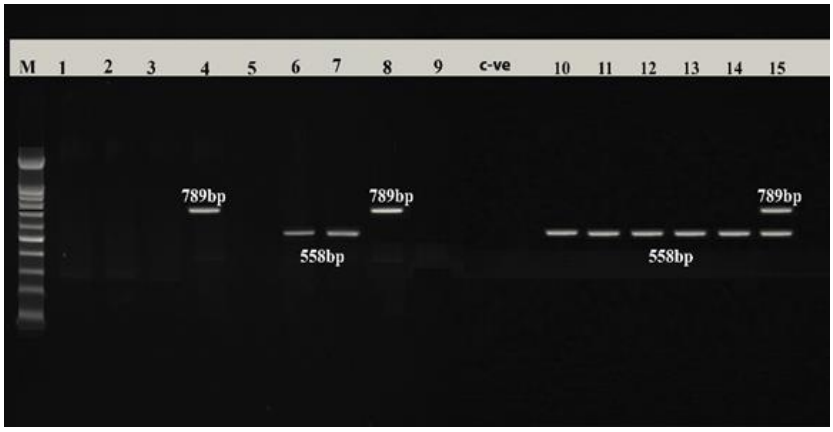


Figure 8 PCR products for intedron genes. Lane M: Ladder; Lane 1; 2; 3; 5 &9 K.pneumoneae free from integron genes. Lanes 6; 7; 10; 11; 12; 13 &14: showed intI gene. Lane 8: showed intII. Lane 15: showed both intI and intII. Lane C-ve: control negative (water)

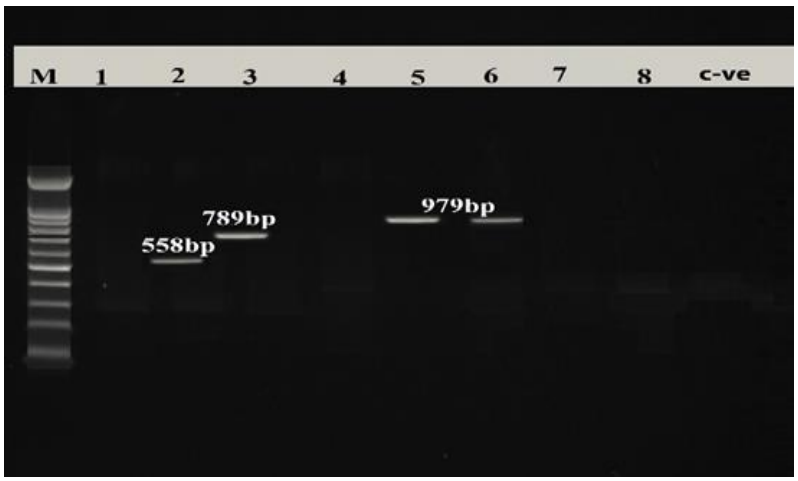


Figure 9 PCR products for intedron genes. Lane M: ladder; Lanes 1;4;7 & 8: *P. aeruginosa* free from integron genes. Lane 2:showed *intI.* Lane 3: showed *intII*. Lanes 5&6 showed *intIII*. Lane C-ve: control negative (water)

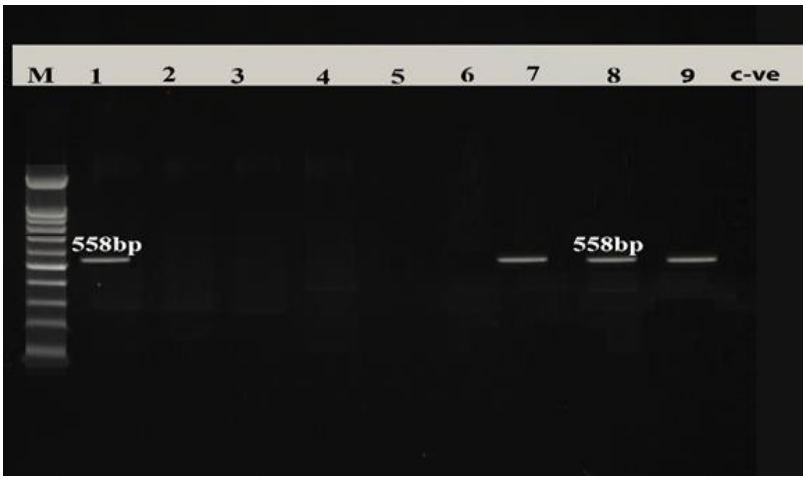


Figure 10 PCR products for integron genes. Lane M: Ladder; Lanes 1: showed *intI in S.zooepidemicus*. lanes 2,3*: S. aooepidemicus* –ve for integron genes. Lanes 7;8&9: *S.equi* showed *intI*. Lane 4;5&6 *S.equi* free from integron genes. Lane C-ve: control –ve (water)

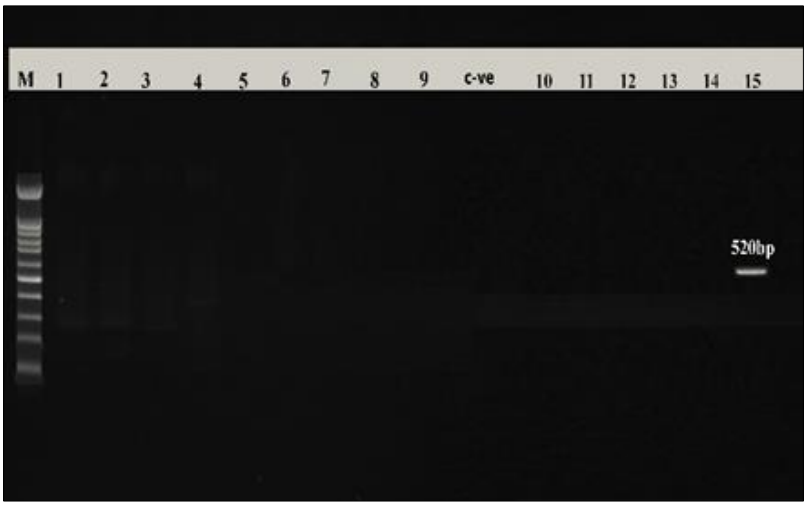


Figure 11 PCR products *for mox-M* genes. Lane M: Ladder; Lanes 15: showed *K.pneumoneae* with mox-*M* gene Lane C-ve: control –ve (water)

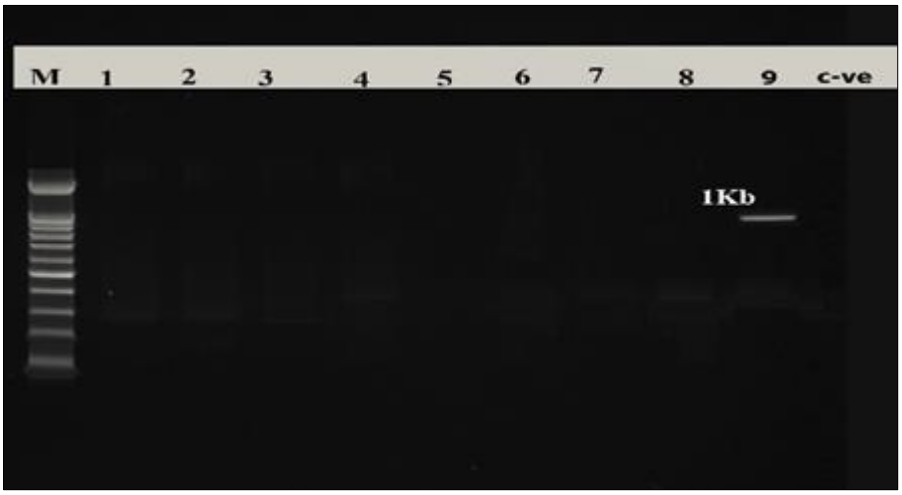
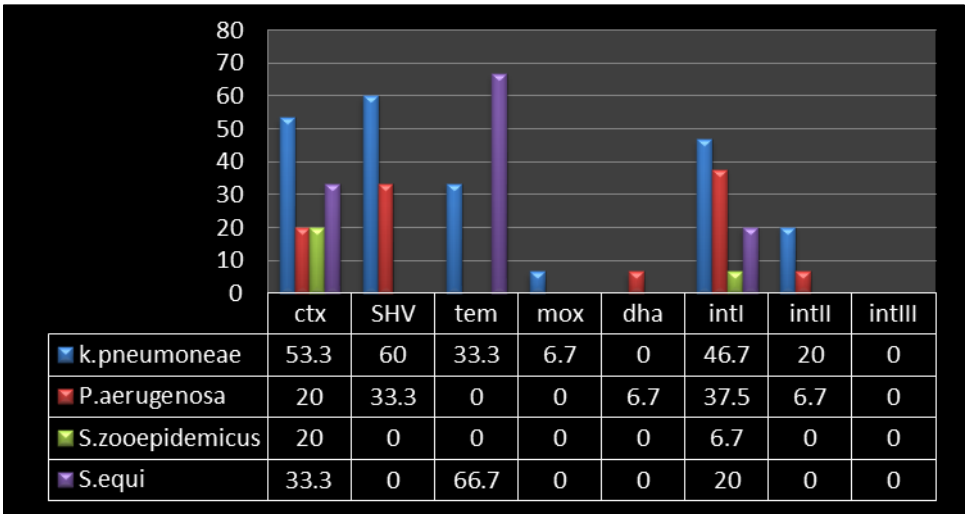


Figure 12 PCR products for DHA-1 genes. Lane M: Ladder; Lanes 9: showed *P.aeruginosa* with DHA-1 gene Lane C-ve: control –ve (water)

Table 6 Occurrence of different antibiotic resistance genes among different clinical isolates

Occurrence of different antibiotic resistance genes among different clinical isolates																	
Type of isolates	**Total No. of isolates**	***ctx***		***shv***		***tem***		***intI***		***intII***		***intIII***		***mox***		***DHA-1***	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
K. pneumoneae	15	8	53.3	9	60	1	7	7	46.7	3	20	0	0	1	6.7	0	0
P.aeruginosa	8	3	20	5	33.3	0	0	3	20	3	37.5	1	6.7	0	0	1	6.7
S.zooepidemicus	3	3	20	0	00	0	0	1	6.7	0	0	0	0	0	0	0	0
S.equi	6	2	33.3	0	00	4	66.7	3	20	0	0	0	0	0	0	0	0



	ctx	SHV	tem	mox	dha	intI	intII	intIII
k.pneumoneae	53.3	60	33.3	6.7	0	46.7	20	0
P.aerugenosa	20	33.3	0	0	6.7	37.5	6.7	0
S.zooepidemicus	20	0	0	0	0	6.7	0	0
S.equi	33.3	0	66.7	0	0	20	0	0

Figure 13 Genotypic profile in different isolates

Another family of β-lactamases is that the SHV enzymes (Sulphydryl variable (shv)-type b-lactamases). The originator of the SHV enzymes, shv-1 was first designated in *Klebsiella pneumoneae*. shv-1 discusses resistance to broad pectrum penicillins. In Germany (1983), a *Klebsiella ozaenae* strain was isolated conceiving an shv-2 enzyme that competently hydrolyzed cefotaxime and, to a smaller range, ceftazidime so far , 450 shv products are known, all being products of shv-1 or shv-2 because the TEM-type enzymes, the foremost of the SHV enzymes be situated as ESBLs (Paterson & Bonomo[17]; Gupta [18]).

The AmpC enzymes are additional important group of broad-spectrum β-lactamases, which are usually prearranged on the chromosome of the many Gram-negative bacteria, comprising Serratia, Citrobacter, and Enterobacter species, where its appearance is usually cannot be urged (Jacoby [21]. The primary bacterial enzyme reported to extinguish penicillin was the AmpC β-lactamase of *Escherichia coli* (Abraham, and Chain [22]. Within the present study just one isolate of *K.pneumoneae* showed presence of mox-M gene and just one isolate *of P.aeruginosa* harbor DHA-1 gene (Fig. 8 & 9 Table 6 & Chart4).

AmpC-type β-lactamases might be harbor on plasmids of bacterial species lacking the chromosomal ampC gene, like Klebsiella spp. (Caroff et al. [23], and one isolate of *P.aeruginosa* harbors DHA-1 gene Mammeri et al. [24] reported that AmpC -lactamases produced some kinetic data on the DHA-1 enzyme for cephaloridine and imipenem only.

Integrons is distributed into two basic groups, the superintegrons and therefore the antibiotic resistance integrons (ARIs) (Cambray et al. [25]; Fluit and Schmitz [26]. Cumulative of antibiotic resistance genes among bacteria can happen by moving genetic elements hedging the ARIs (Cambray et al.[25]; Hall [27]; Hall and Collis [28]. Historically, dissimilar classes of ARIs, classes 1, 2, and three are involved various antibiotic resistance (MAR) phenotypes and are distinct counting on their particular integrase (int) genes (Cambray et al. [25]; Hall [27]. Five isolates of *K.pneumoneae* harbor intI genes (Fig. 9) followed by 4 isolates haven intII and no intIII detected in examined isolates (Table 6& Fig 10). Class 1 integrons (intI) are the foremost widespread and are often established in ESBL producing clinical isolates of Enterobacteriaceae including *K.pneumoniae* (Jones et al. [29]; Machado et al.[30]; Rao et al. [31]; Yao et al. [32]. Class 2 integrons (intII) arise less commonly in ESBL producing *E. coli* and *K. pneumoniae* and lastly, class 3 integrons (intIII) are seldom present in ESBL producing *K. pneumoniae* (Bhattacharjee et al.[33]; Correia et al.[34]; Machado et al. [30].

In *P.aeruginosa*, just one isolate showed intI, 3 isolates harbor intII (Fig.. 12; Table 4& Fig. 13). These results agreed with Yi et al. [35] and one isolate carried *intIII* These result was in concordant to other previous report (Khosravin et al. [36] and agreed with Yi et al. [35] While one isolate of *S.zooepidemicus* showed the presence of intI gene and three isolates of *S.equi* showed intI,while intII and *intIII* wasn't detected in stereptcoccus spp. (Fig.9) Class I integrons can detention and dispense gene cassettes amongst other integron classes. This transmission occurred via the natural conjugation or alteration (Liebert, et al. [37] and Nardelli, et al. [38].

Significant correlation or association of intI genes with ctx-M genes in *K.pneumoneae*, *P.aeruginosa* and streptococcus species was observed, while it had been significant with shv-1 in *K.pneumoneae* and streptococcus species. The significance was at *p<o.05* (Tables 7; 8 & 9).

These results suggest that class I integron genes may have a significant role within the distribution of β-lactamases-encoding genes among clinical β-lactamases-producing *K. pneumoniae ,P.aeruginosa* and Streptcoccus species isolated from equine with respiratory disorders. This development of MDR profiles related to the presence of bla -ctx-M, bla-shv-1, and class I integron genes is disturbing. These results agreed with Ejaz, et al.[39].

4. Conclusion

Table 7 Correlation between antimicrobial resistance genes carriage in 15 ESBL β-lactamases producing of *Klebsiella pneumoneae isolates* using the two-tailed Mann-Whitney U test

Type of gene	Sum of ranks	Mean of ranks:	Expected sum of ranks	Expected mean of ranks	*SD*	*U*-value	Expected *U*-value	*U* value	*Z* Score	*p*-value	*p* < .05
Integron	801	33.28	588	24.5	48.4	75	288	75	4.38	< .00001	Significant
ctx-M	375	15.6				501					
Integron	794	33.08				82		82	4.23	< .00001	Significant
shv	382	15.92				494					
ctx-M	543.5	22.65				332.5		243.5	-0.91	.36282	Non-significant
shv	680.5	28.35				243.5					

Table 8 Correlation between antimicrobial resisttance genes carriage in 6 extended-spectrum β-lactamase producing clinical isolates of P. aeruginosa using the two-tailed Mann-Whitney U test

Type of gene	Sum of ranks	Mean of ranks:	Expected sum of ranks	Expected mean of ranks	*SD*	*U*-value	Expected *U*-value	*U value*	*Z Score*	*p-value*	*p < .05*
Integron	751.5	32.67	540.5	23.5	45.5	53.5	264.5	53.5	4.62	< .00001	Significant
ctx-M	329.5	14.33				540.5					
Integron	552.5	24.02				252.5		252.5	0.25	.802	Non-significant
shv	528.5	15.15				276.5					
ctx-M	348.5	22.65				456.5		72.5	4.20	.36282	Significant
shv	732.5	31.85				72.5					

Table 9 Correlation between antimicrobial resistance genes carriage in 9 extended-spectrum β-lactamase producing clinical isolates of Streptcoccus spp. using the two-tailed Mann-Whitney U test

Type of gene	Sum of ranks	Mean of ranks:	Expected sum of ranks	Expected mean of ranks	*SD*	*U*-value	Expected *U*-value	*U* value	*Z* Score	*p-value*	*p < .05*
Integron	801	33.28	588	24.5	48.4	75	288	75	4.38	< .00001	Significant
ctx-M	375	15.6				501					
Integron	794	33.08				82		82	4.23	< .00001	Significant
shv	382	15.92				494					
ctx-M	543.5	22.65				332.5		243.5	-0.91	.36282	Non-significant
shv	680.5	28.35				243.5					

The presence of β- lactamases genes makes the bacteria immune to several useful pragmatic regimens and results in few therapeutic strategies

The detection of integrons in most of the bacterial isolates promoters the phenomenon of in depth resistance because of these mobile genetic elements Integrons transmit a drug-resistance gene cassette against multiple classes of medicine , and therefore the detection of those mobile gene cassettes in equine may be a keystone of multidrug resistance.

Companion animals predominantly contain bla-ctx-M clusters, which might be animal origin, causing quick changes in drug-resistance epidemiology. ctx-M-1 and shv-1 also are commonly found among human pathogens signifying a standard clonal heredity and a possible ESBL dissemination source in farm and livestock.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors declare no conflict of interest.

Authors' contributions

Soumaya, S. A. El Shafii was responsible for project administration and validation. Soumaya, S. A. El Shafii, Azza, N. F. and Shaimaa, R. A. Abd Elmawgoud cooperated in conceptualization, formal analysis, investigation, methodology, and writing the original draft. Momtaz A. Shahin, and Essam Ibrahim were helpful in data curation, writing, reviewing, and editing. All authors reviewed and approved the last edition of article for publishing in the present journal. Sultan F. Nagati and Hammad O. Hammad: Sample collection, bacteriological examination, writing the paper.

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