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Phenotypic Study on the Bacterial Isolates from Equine with Respiratory Disorders regarding Antimicrobial Drug Resistance

M. Fawzy Nehal[1], M. Osman Kamelia[2], N. F. Azza [1], R. A. Abd Elmawgoud Shaimaa[1], S.A. El Shafii Soumaya[1*], M. A. Shahein[1] and E. M. Ibraheem[1]

[1]*Animal Health Research Institute; Equine Bacterial Diseases Unit, Giza, Egypt*
[2]*Faculty of Veterinary Medicine, Cairo University, Egypt*

*Corresponding author's Email: Dr.soumayaelshafii@yahoo.com; ORCID: 0000-0003-1071-8377

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ABSTRACT

Upper respiratory tract infection and pneumonia in foals are primarily caused by a bacterial infection. Gram-negative bacteria are commonly found in neonatal pneumonia although gram-positive and mixed infections could be accompanied. The current study aimed to detect the different pathogens causing respiratory disorders in the equine, describe the antimicrobial resistance in these pathogens, and determine the types of antimicrobial isolates. A total of 203 different samples were collected from 42 horse foals, 5 adult horses, and 4 donkey foals from June 2019 to April 2020. All samples were subjected to bacteriology analysis and isolated bacteria were analyzed using susceptibility test for different antibacterial agents. The findings indicated that 38 (74.5%) animals were positive for the isolation of bacteria causing respiratory disorders. The most predominant isolates were *Klebsiella pneumoniae subsp.* Pneumoniae followed by *Staphylococcus aureus, Streptococcus equi, Pseudomonas aeruginosa, Streptococcus zooepidemicus, Proteus mirabilis, Rhodococcus equi, Stenotrophomonas maltophilia,* and *Streptococcus mitis*. *Stenotrophomonas maltophilia is* isolated from all organs, including the lungs. All *K. pneumoniae* isolates were sensitive to lomefloxacin, cefotaxime, meropenem, enrofloxacin, neomycin, and chloramphenicol. The *Pseudomonas aerugenosa* (*P. aeruginosa)* is sensitive to aztreonam and 20% of isolates sensitive to Piperacillin-tazobactam. All *Proteus mirabilis* were sensitive to ampicillin-sulbactam, piperacillin-tazobactam, and cefoperazone. *Stenotrophomonas maltophilia* was only sensitive to oxytetracycline and lomefloxacin. *Staphylococcus aureus* was susceptible to Piperacillin-tazobactam (50%), 25% to lomefloxacin; *Streptococcus equi* were sensitive to vancomycin 33.3% while 16.7% to erythromycin and doxycycline, *Streptococcus zooepidemicus* (100%) were sensitive to cefotaxime, meropenem, and doxycycline. All isolates of Enterococcus species were sensitive to penicillin, piperacillin-tazobactam, and lomefloxacin. Moreover, *Rhodococcus equi* (one isolate) was only sensitive to clarithromycin. The antimicrobial susceptibility test illustrated the presence of multidrug-resistant and pan-drug resistant isolates which proved the indiscriminate and extensive use of antibiotics. In conclusion, resistance monitoring data and risk assessment identified several direct and/or indirect predisposing factors to be potentially associated with MDR development in the equine health sector of Egypt. The predisposing factors may be attributed to insufficient veterinary healthcare, monitoring, and regulatory services, in addition to the intervention of animal health service providers, and/ or farmers' lack of knowledge about drugs. The misuse and overuse of antibiotics have led to the evolution of antibiotic-resistant bacteria in equine in Egypt.

Keywords: Antimicrobial agents, *Klebsiella pneumoniae, Streptococcus zooepidemicus*.

INTRODUCTION

Substantial morbidity and mortality in foals are commonly due to lower and upper respiratory tract infections that is attributed to the interactions between innate immunologic factors and management risk factors (Galvin and Corley 2010). Neonatal pneumonia is commonly caused by Gram-negative bacteria, although Gram-positive and mixed infections do occur. The development of pneumonia can be complex in the foal as it can be caused by multiple organisms-viruses, bacteria, and even internal parasites (Léguillette et al., 2002).

Pneumonia in foals is primarily caused by a bacterial infection and among all isolates, *Streptococcus zooepidemicus* and *Rhodococcus equi* are the most important Gram-positive bacteria. These organisms can be obtained from pure culture or a pleurimicrobial infection. Several other aerobic bacterial species may also occur, including, *Actinobacillus* spp, *Bordetella bronchiseptica, Escherichia coli, Klebsiella pneumoniae*, *Pasteurella* spp, *Pseudomonas* spp, *Salmonella* spp., and *Staphylococcus* spp. (Welsh, 1984). *Klebsiella* spp. is concerned as a common cause of bacterial pneumonia in horses, but few reports describe the clinical presentation and disease progression (Estell et al., 2016). Strangles is a highly contagious disease caused by the abscess-forming bacteria *Streptococcus equi*, mainly foals, and horses of any age can also be infected. It seems to cause severe and economically important respiratory disease in horses (Erol et al., 2012; Rush, 2014). One Health (OH) is a vital conceptualization when the intervention that occurs

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between humans, animals, and the environment is considered. The horses' footprint on the well-being of the environment and humans forces the incorporation of the horse in any roadmap to achieve OH (Lönker et al., 2020). Antimicrobial resistance in equine medicine has received relatively limited attention which encourages individuals to indulge in this endeavor to throw light on the situation of microbial resistance in the bacterial community allocated in the respiratory tract of equines.

The aim of this study was to detect the rate of different pathogens causing respiratory disorders in equine and describe the rate of antimicrobial resistance in pathogens, and to determine the type of antimicrobial isolates.

MATERIALS AND METHODS

A database search was performed of submissions to Equine Bacterial Diseases Unit (EBDU) within time interval June 2019 to April 2020 for the bacterial culture of samples from foals**,** adults, and donkeys (Table 1). Samples were enriched on buffer peptone water and incubated at 37C for 18-24 hours. The enriched samples were cultured on duplicated plates blood agar and staph strep media with strep supplement and 5% sheep blood (UK standard, 2014a). Also, the enriched samples were cultured on mannitol media or Baird Barker media, and plates incubated at 37°C for 24 hours **(**UK standard, 2014b), and on Tinsdale media at 37°C for 24-72 hours aerobically (UK standard, 2014c). Small Colonies showed β hemolysis or α hemolysis, which were examined for catalase test and oxidase test, golden yellow on mannitol or black colonies with hallow zone on Baird Barker. Non-hemolytic colonies and Tinsdale agar showed small dark brown colonies. Furthermore, enrichment samples were cultured on blood agar, MacConkey agar (UK standard, 2014d), and pseudomonas agar (CN media, UK standard, 2015). All suspected colonies were further biochemical identified using S.R.O. GP24 and S.R.O. GN24 kits (diagnostics.S.R.O.^{TM}).

Susceptibility test for different isolates against antimicrobial agents

The type, symbol, and concentration of antimicrobial agent used were illustrated in supplementary Table 2. Each culture was cultured onto a non-inhibitory agar medium. After incubation at 35°C overnight, four or five well-isolated colonies were selected and transferred to Mueller-Hinton broth and vortex thoroughly, incubated the broth at 35°C, and then adjusted the turbidity (0.5 McFarland standard tube). The procedure continued by using a sterile cotton swab, dipping into the suspension, and culturing over the entire surface of the medium, and rotating the plate approximately 60 degrees after each application. This procedure was repeated three times to ensure an even distribution of the inoculum, CLSI, 2012). The antimicrobial discs were applied to the plates and incubated at 35°C for 16 to 18 hours. The diameter of the zones of complete inhibition was measured. Interpretation of results was recorded according to CLSI (2017). Pareto chart was used to demonstrate the contribution of each type of bacteria in respiratory infections. It was conducted using QI Macros software that has been loaded to the startup directory of Microsoft Office Excel 2013.

Table 1. Type and numbers of samples collected from private farms in Egypt from June 2019 to April 2020

Period of collection	Type of animals	Total number of animals	Type of samples	Number of each type of samples
6-12/2019	Foals (20 days-3years)	9	Nasal swabs	9
			Feces	1
		12	Internal organs*	81
	Adults	2	Nasal swabs	-
			Feces	-
			Internal organs*	14
Subtotal		23		105
1-4/2020	Foals (20 days-3years	10	Nasal swabs	10
			feces	10
		11	Internal organs*	52
	Adults	3	Nasal swabs	-
			feces	-
			Internal organs*	18
Subtotal		24		90
3/2020	Donkey's foal	4	Nasal swabs	4
			Feces	4
Subtotal		4		8
Total		51		203

*Internal organs: Lung, trachea, liver, spleen, heart, kidney, and Intestine

Table 2. List of antimicrobial disks used for antibiotic sensitivity test

Serial	Antimicrobial agents	Symbol	Concentration (µg)
1	Penicillin	P	10
2	Oxacillin	OX	1
3	Ampicillin	AMP	10
4	Ampicillin-sulbactam	SAM	20
5	Ampicillin-clavulanic acid	AMC	30
6	Piperacillin-tazobactam	TZP	110
7	Cephalexin	CL	30
8	Cephradine	CE	30
9	Cefotaxime	CTX	30
10	Cefoperazone	CFP	75
11	Cefquinome	CEQ	30
12	Meropenem	MEM	10
13	Aztreonam	ATM	300
14	Clarithromycin	CLR	10
15	Erythromycin	E	15
16	Oxytetracycline	OT	30
17	Chloramphenicol	C	30
18	Norfloxacin	NO	10
19	Ofloxacin	OFX	5
20	Lomefloxacin	LOM	10
21	kanamycin	K	30
22	Novobiocin	NV	30
23	Streptomycin	S	10
24	Neomycin	N	10
25	Amikacin	AK	30
26	Linezolid	LZD	30
27	Clindamycin	DA	2
28	Vancomycin	VA	30
30	Amoxicillin- clavulanic acid	Amox-clav	30
31	Doxycycline	D	30

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RESULTS AND DISCUSSION

Recently, an obvious growing interest in equine breeding and industry in Egypt has been observed, which has a great impact on the healthcare of horses as a whole, and particularly on their respiratory infection.

Pneumonia in equine is most frequently caused by Gram-positive bacteria which may be accompanied by Gram-negative (Estell et al., 2016). Out of 51 horses (203 samples), 38 (74.5%) animals were positive for isolation of bacteria causing respiratory disorders. The rates of different bacteria isolated from different samples in foals and adults are illustrated in Table 3. As can be seen, 36 isolates were obtained (17.7%) which was less than the obtained of Toombs-Ruane et al. (2015, 63%). These different results may be attributed to the different environmental or climatic conditions. About 23.8% of the microorganisms were isolated from samples collected during the period June 2019 to December 2019 and 12.2% collected during the period January 2020 to April 2020. Samples of diseased donkey's foals showed no bacteria. The decrease in the isolation rate may be attributed to slight care of the hygienic management (Saastamoinen et al., 2015).

Klebsiella pneumoniae (*K. pneumoniae*) showed the highest rate of isolation regarding internal organs (26.3%, Table 4), followed by *Staphylococcus aureus* (*S. aureus)*, *Streptococcus equi subsp. Equi (S. equi subsp. Equi)*, and *Pseudomonas aeruginosa* (*P. aeruginosa)* (10.5%, 4.5%, and 3.8% respectively). Also, *Proteus mirabilis (P. mirabilis*) and *Streptococcus equi subsp. zooepidemicus* (*S. zooepidemicus*) were isolated at the same rate of 2.3%. Nasal samples of foals showed one isolate *S. aureus* and one isolate of Rhodococcus equi (*R. equi)* isolated from fecal samples. In adult horses, only Enterococcus species isolated from internal organs had a rate of 6.2% (Table 5). *Klebsiella* spp are a common cause of bacterial pneumonia but cases are not well-described in the literature, as Estell et al. (2016) stated that mixed infection (polymicrobic infection) is more common in older foals, in which *S. zooepidemicus* is the most predominant, followed by *Actinobacillus suis*, and Pasteurella spp. The obtained results of *E. coli*, *Klebsiella pneumoniae* were on the contrary with Wood et al. (2005) who found that *S. zooepidemicus* and *S. pneumoniae* are the most common ones followed by *Actinobacillus, Pasteurella*, and *Mycoplasma equirhinis*.

Stenotrophomonas maltophilia (S. maltophilia) is isolated from all organs, including the lung, for the first time in Egypt . Recently, *S. maltophilia* is being recorded as a human nosocomial infection causing pneumonia with increasing incidence and has not previously been associated with lower airway disease in the horse. However, Winther et al. (2009) reported the clinical findings, laboratory diagnosis, and response to treatment of seven cases of respiratory infection with *S. maltophilia* in horses.

Table 6 and Figures 1 and 2 showed the rate of single and mixed infection in dead animals, where 5 animals showed mixed infection with *K. pneumoniae* and *S. aureus* (13.1%), also *S. aureus* with *Ps. aeruginosa* was a mixed infection in 7.9% of cases. The *K. pneumoniae* indicated the highest rate of single infection (26.3%). *Stenotrophomonas maltophilia* (2.6%) as it isolated from all organs, including lung, is isolated in Egypt for the first time. These obtained results were in agreement with those reported by Wilson (2001).

Antimicrobial agent's action occurs by interrupting specific metabolic functions within bacterial cells. There are four primary targets for antimicrobial action, including disruption of cell wall synthesis, inhibition of DNA/RNA synthesis, inhibition of protein biosynthesis, or interference with a crucial metabolic pathway (Roberts, 2005). There has been a scarcity in the studies investigating the antimicrobial resistance profile in the bacteria that have been isolated from the respiratory tract of horses (Johns and Adams, 2015; Álvarez–Narváez et al., 2020; Lönker et al., 2020).

The *K. pneumoniae*. isolates were sensitive to lomefloxacin, cefotaxime, meropenem, enrofloxacin, neomycin, and chloramphenicol (15.4%, 13.3%, 13.3%, 6.7%, 6.7%, and 6.7%, respectively, Table 7). Fluoroquinolones are predominantly active against Gram-negative aerobes, including Enterobacteriaceae and *Pseudomonas aeruginosa*, against *Mycoplasma* spp., *Rickettsia* spp., and *Ehrlichia* spp. They have limited Gram-positive coverage, except for many *Staphylococcus* spp. (Haggett and Wilson, 2008). Enrofloxacin is the only fluoroquinolone presently in clinical use in horses. Although different doses have been reported in the literature for other fluoroquinolones, there is a lack of reliable data (Bousquet-Melou et al., 2002; Davis et al., 2006; Fernandez-Varon et al., 2006).

The *P. aeruginosa* is sensitive to aztreonam (100%) and 20% of isolates sensitive to Piperacillin-tazobactam. The monobactams do not have any activity against Gram-positives or anaerobic bacteria. However, they are highly effective against certain Gram-negative bacteria, especially the enteric Gram-negative rods and can be used for *Pseudomonas aeruginosa* (Chirality, 2012). All *Proteus mirabilis* (3 isolates) were sensitive to ampicillin-sulbactam, piperacillin-tazobactam, and cefoperazone (100%). Only, 33.3% of isolates were sensitive to enrofloxacin, *Stenotrophomonas maltophilia* (one isolate) was sensitive to oxytetracycline (Table 7). These results were in accordance with O'Hara et al. (2000) and Deredjian et al. (2016). As can be seen in Table 7, *S. aureus* was susceptible to Piperacillin-tazobactam (50%) and 25% to lomefloxacin. It was recorded that the bactericidal activity of piperacillin/tazobactam was noticed 1 hour after drug administration for *S. aureus, E. coli, and P. aeruginosa* (Lemmen et al., 2004). Moreovr, it was found that *S. equi* (causing strangles) was sensitive to doxycycline and erythromycin (16.7%). *S. zooepidemicus* was sensitive to cefotaxime, meropenem, and doxycycline (100%), which supported the findings of Lemmen et al. (2004).

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R. equi (one isolate) was sensitive only to Clarithromycin (Table 7). Pneumonia caused by *R. equi* is a major health problem for equine industries on a worldwide basis. A combination of macrolide with rifampin is recommended for the treatment of infection caused by *R. equi* (according to the *in-vitro* activity) when there are no highly effective preventatives (Gigue`re et al., 2011). Heatmap analysis showed the intensity of antibiotic resistance of different isolates based on the percentage of resistance (Figure 3). Each row indicates the type of isolate and each column represents the type of antimicrobial agents most of which showed 100% resistance. The phenotypic resistance pattern, prevalence, and diversity of the four Gram-ve bacteria species *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, and *Stenotrophomonas maltophilia* isolates are recorded in Table 8. They were tested for their resistance phenotypic profile against 25 antibiotics representing 9 classes. They were resistant to the 15 antibiotics. Moreover, the five Gram-positive bacteria species isolated from the respiratory tract (*S. aureus, S. equi,* and *S. zooepidemicus*), feces *Enterococcus* spp., and one isolate of *R. equi* were tested for their phenotypic resistance patterns against 27 antibiotics representing 11 classes (Table 9).

This diversity of Gram-negative bacteria and Gram-positive bacteria isolated from the respiratory tract reflect the capacity of 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., 2012; Garza-Cervantes et al., 2020). Highly resistant Gram-negative bacteria were *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* have become very difficult to treat pathogens (Boucher et al., 2009) and are, therefore, considered as the ESKAPE pathogens (Pendleton et al., 2013), including some Gram-positive bacteria, such as *Staphylococcus*, *S.equi*, and *S.zooepidemicus* as well as *Enterococcus* species (Coates et al., 2002; Smith and Romesberg, 2007; Hegreness et al., 2008).

In the present study, 11 isolates of *K. pneumoneae* were typed as multidrug resistance (MDR) and 4 isolates were pan-drug resistance (PDR), all isolates of *P. aeruginosa* were PDR while all isolates of *P. mirabilis* and *S. maltophilia* were MDR (Table 10). While all isolates of Gram-positive isolates were PDR except the two isolates of *S. zooepidemicus* which were MDR (Table 11). Antibiotic resistance (El Zowalaty et al., 2015; Magiorakos et al., 2012) is classified into MDR which is not susceptible to at least one representative from each of three categories of selected antimicrobial compound families (El Zowalaty et al., 2015; Fodor et al., 2020). Extreme or extensively drug-resistant (XDR) is not susceptible to at least a single representative of all but very few categories of antimicrobial compound families. The PDR is not susceptible to any of the tested or empirical representatives of all known antimicrobial compound families (El Zowalaty et al., 2015).

The MDR and PDR isolates are inconsistent in medical literature, disqualifying reliable comparison of data. In order to reach a standardized definition, we applied the multidrug resistance definition from human medicine (Magiorakos et al., 2012). This adaption was limited by the unattainability of certain susceptibility results and differing antimicrobial agents in human and veterinary medicine. Therefore, the establishment of a standard definition of MDR bacteria in veterinary medicine should be supported.

Table 3. Rate of different bacteria isolated from different samples collected from private equine farms during the period from June 2019 to April 2020

Period of sample collected	Number of animals	Age of horses	Type of samples	Total Number of samples	Results		
					Number of positive samples	Number of negative samples	% of positive results
6-12-2019	21	Foals (20 days- 3 years)	Internal organs	81	18	63	22.2
			Nasal	9	0	9	0
			Fecal	1	1	0	0
	2	Adults over 3 years	Internal organs	14	6	8	42.9
			Nasal	0	0	0	0
			Fecal	0	0	0	0
Subtotal	23			105	25	80	23.8%
1-4-2020	11	Foals	Internal organs	52	11	41	21.2
			nasal	10	0	10	0
			Fecal	10	0	10	
	3	Adults	Internal organs	18	0	18	0
			Nasal	-	-	-	
			Fecal	-	-	-	
Subtotal	14			90	11	73	12.2%
1-4/2020	4	Donkey`s foal	Internal organs	0	0	0	0
			Nasal	4	0	4	0
			Fecal	4	0	4	0
		Adult donkeys	Internal organs	0	0	0	0
			Nasal	0	0	0	0
			Fecal	0	0	0	0
Subtotal				8	0	8	
Total	50	-	-	203	36	161	17.7%

* Percentage calculated according to total number of each type of samples

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Table 4. Number and type of different bacteria isolated from different samples of foals during the period from June 2019 to April 2020.

Type of samples	Number of samples	Type of isolated bacteria	Number of isolated organisms	Percentage*
Nasal swabs	23	*Staph. aureus*	1	4.3
Fecal swabs	15	*Rhodococcus equi*	1	6.7
Internal organs	133	*Stenotrophomonas maltophilia*	1	0.8
		Staph. aureus	8	6.01
		Streptococcus. zooepidemicus	3	2.3
		Streptococcus equi subsp. equi	6	4.5
		Streptococcus mitis	1	0.8
		Pseudomonas aeruginosa	5	3.8
		Klebsiella pneumoniae	15	11.2
		Proteus mirabilis	3	2.3
Total	171	-	44	61.9

* Percentage calculated according to the total number of samples

Table 5. Number of different bacteria isolated from different samples in adult horses' equine during the period from June 2019 to April 2020.

Type of samples	Number of samples	Type of isolated bacteria	Number of isolated organisms	Percentage*
Internal organs	32	*Enterococcus spp.*	2	6.2%
Total	32	-	2	6.2%

* Percentage calculated according to the total number of samples

Table 6. Rate of isolated bacteria among infected horses during the period from June 2019 to April 2020.

Type of bacteria	Type of positive organs	Number of isolates in IO of foals	Number of isolates in Fecal swab	Number of isolates in Nasal swab	Number of isolates in IO of adults	Number of positive animals	Rate of bacterial isolates*
Rhodococcus equi	-	0	1	0	0	1	2.6%
Klebsiella pneumoniae	All organs	10	0	0	0	10	26.3%
Staphylococcus aureus	All organs	3	0	1	0	4	10.5%
Klebsiella pneumoniae + Staphylococcus aureus	Lung + trachea	5	0	0	0	5	13.1%
Streptococcus equi subsp. equi	Lung + trachea	3	0	0	0	3	7.9%
Streptococcus equi +Pseudomonas aeruginosa	All organs	3	0	0	0	3	7.9%
Streptococcus zooepidemicus	Lung	3	0	0	0	3	7.9%
Pseudomonas aeruginosa	All organs	2	0	0	0	2	
Streptococcus mitis	Lung, liver, spleen	1	0	0	0	1	2.6%
Stenotrophomonas maltophilia	All organs	1	0	0	0	1	2.6%
Proteus mirabilis	All organs	3	0	0	0	3	7.9%
Enterococcus species	All organs	0	0	0	2	2	5.3%
Total		34	1	1	2	38	89.5

* Rate of bacterial isolates was calculated according to the total Number of animals (38), IO: Internal organs

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Table 7. Susceptibility antimicrobial agents for different bacterial isolates.

Antimicrobial agents	Gram negative bacteria								Gram negative									
	Klebsiella. pneumoniae		*Pseudomonas aeruginosa*		*Proteus mirabilis*		*Stenotrophomonas maltophilia*		*Staphylococcus aureus*		*Streptococcus equi*		*Streptococcus zooepidemicus*		*Enterococcus species*		*Rhodococcus equi*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
β-lactam																		
Penicillins																		
Penicillin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	100	0	0
Oxacillin	-	-	0	0	0	0	0	0	0	0	0	0	-	-	0	0	0	0
Ampicillin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
β-Lactam/β-Lactamase Inhibitor Combinations																		
Ampicillin-sulbactam	0	0	0	0	3	100	0	0	0	0	0	0	0	0	0	0	0	0
Ampicillin-clavulanic acid	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piperacillin-tazobactam	0	0	1	20	3	100	0	0	4	50	-	-	0	0	2	100	0	0
Cephems																		
Cephalexin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cephradine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cefotaxime	0	0	0	0	0	0	0	0	0	0	0	0	3	100	0	0	0	0
Cefoperazone	2/15	13.3	0	0	3	100	0	0	0	0	0	0	-	-	0	0	0	0
Cefquinome	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monobactam																		
Meropenem	2/15	13.3	0	0	0	0	0	0	0	0	0	0	3	100	0	0	0	0
Aztreonam	0	0	5	100	0	0	0	0	-	-	-	-	-	-	0	0	0	0
Non β-lactam																		
Macrolides																		
Clarithromycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100
Erythromycin	0	0	0	0	0	0	0	0	0	0	1	16.7	0	0	0	0	0	0
Tetracyclines																		
Oxytetracycline	0	0	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0
Doxycycline	-	-	-	-	-	-	-	-	0	0	1	16.7	3	100	0	0	0	0
Fluoroquinolones																		
Norfloxacin	0	0	0	0	1	33.3	0	0	0	0	0	0	0	0	0	0	0	0
Ofloxacin	0	0	0	0	0	0	0	0	1	12.5	0	0	0	0	0	0	0	0
Lomefloxacin	2/13	15.4	0	0	0	0	0	0	2	25	0	0	0	0	2	100	0	0
Enrofloxacin	1/15	6.7	0	0	1	33.3	0	0	/-	-	-	-	-	-	-	-	0	0
Aminoglycosides																		
Kanamycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phenicols																		
chloramphenicol	1/15	6.7																
Novobiocin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptomycin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neomycin	1/15	6.7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amikacin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxazolidinones																		
Linezolid	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0
Lincosamides																		
Clindamycin	-	-	-	-	-	-	-	-	0	0	0	0	0	0	0	0	0	0
Glycopeptides																		
Vancomycin	-	-	-	-	-	-	-	-	0	0	2	33.3	0	0	0	0	0	0

(-): Not applied

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Table 8. Phenotypic resistance pattern of Gram-negative bacteria isolated from all samples.

Bacterial isolates	β-lactam / Penicillins			β-Lactam/β-Lactamase Inhibitor Combinations			Cephems					Monobactam		Macrolides	Tetracyclines		Phenicols	Fluoroquinolones				Aminoglycosides				
	Penicillin	Oxacillin	Ampicillin	Ampicillin-sulbactam	Ampicillin - clavulanic acid	Piperacillin-tazobactam	Cephalexin	Cephradine	Cefotaxime	Cefoperazone	Cefquinome	Meropenem	Aztreonam	Clarithromycin	Oxytetracycline	Doxycycline	Chloramphenicol	Norfloxacin	Ofloxacin	Lomefloxacin	Enrofloxacin	kanamycin	Novobiocin	Streptomycin	Neomycin	Amikacin
Klebsiella pneumoniae	R		R	R	R	R	R	R	R	S	R	S	R	R	R		S	R	R	S	S	R	R	R	S	R
Klebsiella pneumonia	R		R	R	R	R	R	R	R	S	R	S	R	R	R		R	R	R	S	R	R	R	R	R	R
Klebsiella pneumonia	R		R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R
Klebsiella pneumonia	R		R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia			R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia			R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia			R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia			R	R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia				R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia				R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia				R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia				R	R	R	R	R	R	R	R	R	R	R	R		R	R	R	R	R		R	R	R	R
Klebsiella pneumonia				R	R	R	R	R	R	R	R	R	R	R	R		R	R	R		R		R	R	R	
Klebsiella pneumonia				R	R	R	R	R	R	R	R	R	R	R	R		R	R	R		R		R	R	R	
Klebsiella pneumonia				R		R	R	R	R	R	R	R	R	R	R		R	R	R				R	R	R	
Pseudomonas aeruginosa	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R		R	R	R	R		R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R		R	R	R	R		R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R		R	R	R	R		R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R		R	R	R	R		R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R		R	R	R	R		R	R	R	R	R
Proteus mirabilis	R	R	R	S	R	S	I	R	R	S	R	R	R	R	R		R	S	R	R		R	R	R	R	R
Proteus mirabilis	R	R	R	S	R	S	I	R	R	S	R	R	R	R	R		R	R	R	R		R	R	R	R	R
Proteus mirabilis	R	R	R	S	R	S	R	R	R	S	R	R	R	R	R		R	R	R	R		R	R	R	R	R
Stenotrophomonas. maltophilia	R			R	R			R			R	R	R		S					S						R

R: Resistant; S: Sensitive; I: intermediate.

Table 9. phenotypic resistance pattern of Gram-positive bacteria isolated from different samples

Bacterial isolates	β-lactam			β-Lactam/β-Lactamase Inhibitor Combinations			Cephems					Monobactam	Macrolides		Tetracyclines		Phenicols	Fluoroquino-lones			Aminoglycosides					Oxazolidinone	Lincosamide	Glycopeptide
	Penicillins																											
	Penicillin	Oxacillin	Ampicillin	Ampicillin-sulbactam	Ampicillin - clavulanic acid	Piperacillin-tazobactam	Cephalexin	Cephradine	Cefotaxime	Cefoperazone	Cefquinome	Meropenem	Clarithromycin	Erythromycin	Oxytetracycline	Doxycycline	Chloramphenicol	Norfloxacin	Ofloxacin	Lomefloxacin	kanamycin	Novobiocin	Streptomycin	Neomycin	Amikacin	Linezolid	Clindamycin	Vancomycin
Staphylococcus aureus	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R		R	S	S	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R		R	R	S	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	S	R		R	R	R	R	R		R	R		R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	S	R		R	R	R	R	R		R	R		R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R		R	R	R	R	R		R	R		R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R		R	R	R	R	R		R	R		R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R		R	R	R	R	R		R	R		R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R		R	R	R	R	R		R	R		R	R	R	R	R	R	R	R	R	R	R
Streptococcus equi	R	R	R	R	R		R	R	R	R	R	S	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	S
Streptococcus equi	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S
Streptococcus equi	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptococcus equi	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptococcus equi	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptococcus equi	R	R	R	R	R		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptococcus zooepidemicus	R		R	R	R		R	R	S		R	S	R	R	R	S		R	R	R	R	R	R		R		R	R
Streptococcus zooepidemicus	R		R	R	R		R	R	S		R	S	R	R	R	S		R	R	R	R	R	R		R		R	R
Streptococcus zooepidemicus	R		R	R	R		R	R	S		R	S	**R**	**R**	**R**	**S**		R	R	I	R	R	R		R		R	R
***Enterococcus* species**	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R		R	R	S	R	R	R	R	R	R	R	R
***Enterococcus* species**	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R		R	R	S	R	R	R	R	R	R	R	R
Rhodococcus equi	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

R: Resistant, S.: Sensitive, I: intermediate

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Table 10. Multidrug resistance profiles of the Gram negative bacterial species isolated from respiratory tract of equines

Number of resistant AB	Number of resistant AB classes	Antibiotics	Number of isolates	Type of resistance	Total number of Isolates (n = 24)
9	5	P, AMP, S, AMC, TZP, CL, CTX, CEQ, CLR	1	MDR	15 (*K.pneumoniae*)
12	6	P, AMP, AMC, TZP, CL, CTX, CEQ, C, ENR, NV, S, N	1	MDR	
16	8	P, AMP, AMC, TZP, CL, CTX, CEQ, MEM, ATM, CLR, C, LOM, ENR, NV, S, N	1	MDR	
16	8	P, AMP, AMC, TZP, CL, CE, CEP CEQ, MEM, ATM, CLR, C, LOM, ENR, NV, N	1	MDR	
18	8	SAM, TZP, CL, CE, CTX, CEP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, NV, S, N	1	MDR	
20	8	SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, ENR, NV, S, N	2	MDR	
20	8	SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, ENR, NV, S, N	2	MDR	
22	8	SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	2	MDR	
23	9	AMP, SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, ATM, CLR, E, OT, C, NO, OFX, LOM, ENR, NV, S, N, AK	4	PDR	
23	9	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, CLR, OT, C, NO, OFX, LOM, K, NV, S, N, AK	2	PDR	5 (*P.aeruginosa*)
22	9	P, OXA, AMP, SAM, AMC, TZP, CL, CE, CTX, CEP, CEQ, MEM, CLR, OT, C, NO, OFX, K, NV, S, N, AK	2	PDR	
21	9	P, OXA, AMP, SAM, AMC, CL, CE, CTX, CEP, CEQ, MEM, CLR, OT, C, NO, OFX, K, NV, S, N, AK	1	PDR	
17	8	P, OXA, AMP, AMC, CE, CTX, CEQ, MEM, ATM, CLR, OT, C, K, NV, S, N, AK	1	MDR	3 (*P. mirabilis*)
16	8	P, OXA, AMP, AMC, CTX, CEQ, MEM, ATM, CLR, OT, C, K, NV, S, N, AK	2	MDR	
6	8	SAM, AMC, CE, CEQ, MEM, ATM	1	MDR	1 *S. maltophilia)*

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.

Table 11. Multidrug resistance profiles of the Gram +ve bacteria species isolated from respiratory tract and feces of equines

Number of resistant AB	Number of resistant AB classes	Antibiotics	Number of isolates	Type of AMR	Number of isolates (n = 19)
24	11	P, OXA, Amp, SAM, AMC, CFX, CE, CPZ, TZP, CEQ, MEM, CLR, OXT, NOR, K, NV, DO, NO, S, N, AK, DA, VA, LZD	1	PDR	8 (*S. aureus*)
25	11	P, OXA, Amp, SAM, AMC, CFX, CE, CPZ, TZP, CEQ, MEM, CLR, OXT, NOR, OFX, K, NV, DO, NO, S, N, AK, DA, VA, LZD	1	PDR	
25	11	P, OXA, Amp, SAM, AMC, CFX, CPZ, TZP, CEQ, MEM, CLR, **E**, OXT, NOR, OFX, LOM, NV, DO, NO, S, N, AK, DA, VA, LZD	3	PDR	
25	11	P, OXA, Amp, SAM, AMC, CFX, CPZ, TZP, CEQ, MEM, CLR, OXT, NOR, OFX, LOM, K, NV, DO, NO, S, N, AK, DA, VA, LZD	2	PDR	
26	11	P, OXA, Amp, SAM, AMC, CFX, CPZ, TZP, CEQ, MEM, CLR, E, OXT, NOR, OFX, LOM, K, NV, DO, NO, S, N, AK, DA, VA, LZD	1	PDR	
23	8	P, OXA, AMP, SAM, AMC, CE, CTX, KF, CEP, CEQ, CLR, E, OTX, C, NOR, OFX, LOM, K, NV, S, N, AK, DA	1	MDR	6 (*S. equi* Equi)
26	11	P, OXA, AMP, SAM, AMC, CE, CTX, KF, CEP, CEQ, MEM, CLR, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA	1	PDR	
27	11	P, OXA, AMP, SAM, AMC, CE, CTX, KF, CEP, CEQ, MEM, CLR, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA, VA	4	PDR	
18	9	P, AMP, SAM, AMC, CE, CF, CEQ, CLR, E, OTX, NOR, OFX, K, NV, S, AK, DA, VA	1	MDR	3 S. Zooepidemicus)
19	9	P, AMP, SAM, AMC, CE, CF, CEQ, CLR, E, OTX, NOR, OFX, LOM, K, NV, S, AK, DA, VA	2	MDR	
24	11	OXA, AMP, SAM, AMC, CE, CTX, KF, CEP, CEQ, MEM, ATM, CLR, E, OTX, DO, NOR, OFX, K, NV, N, AK, LNZ, DA, VA	2	PDR	2 (E*nterococcus*)
26	11	P, OXA, AMP, SAM, AMC, CE, CTX, KF, CEP, CEQ, MEM, E, OTX, DO, C, NOR, OFX, LOM, K, NV, S, N, AK, LNZ, DA, VA	1	PDR	1 *R.equi*

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

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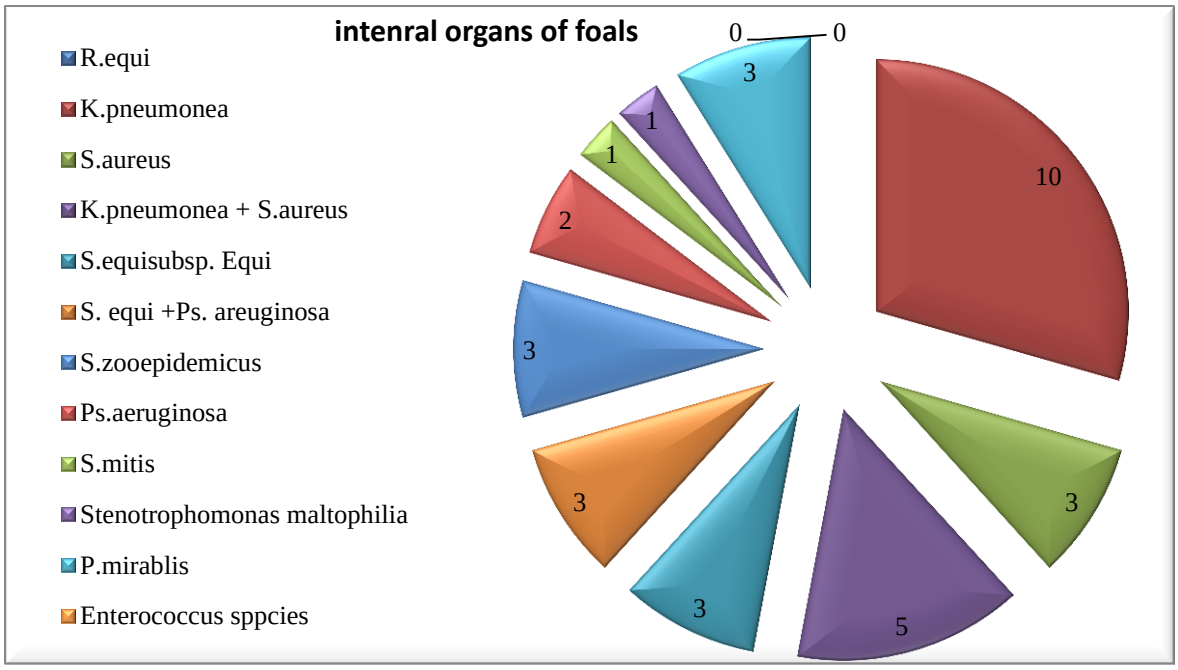

Figure 1. Number and type of isolates in internal organs of dead foals

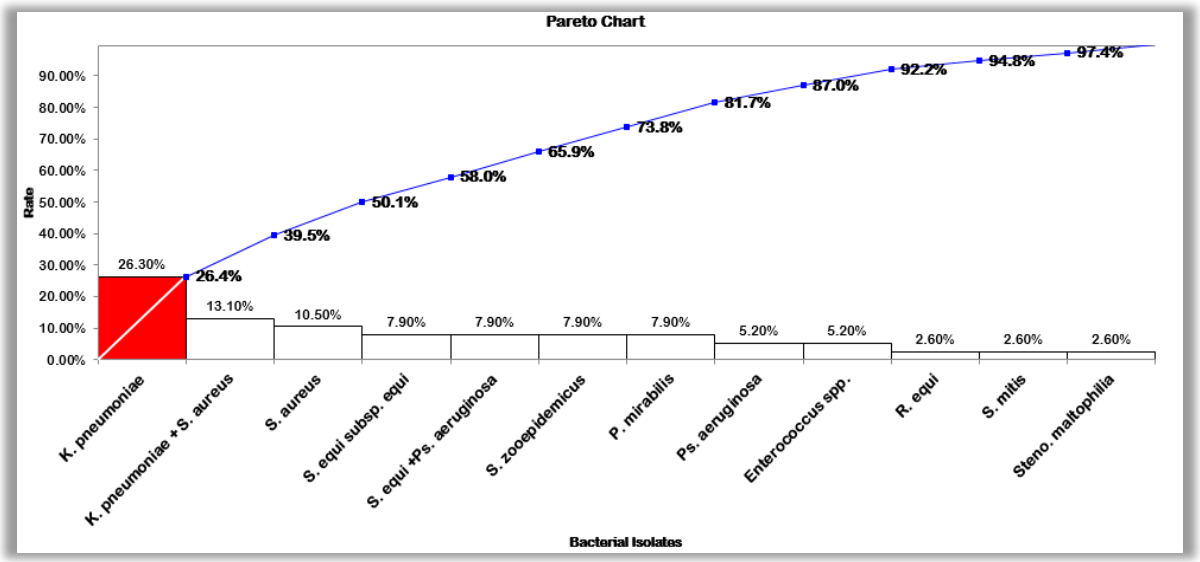

Figure 2. Pareto chart showing the rate of participation of different bacteria in respiratory infections in equine

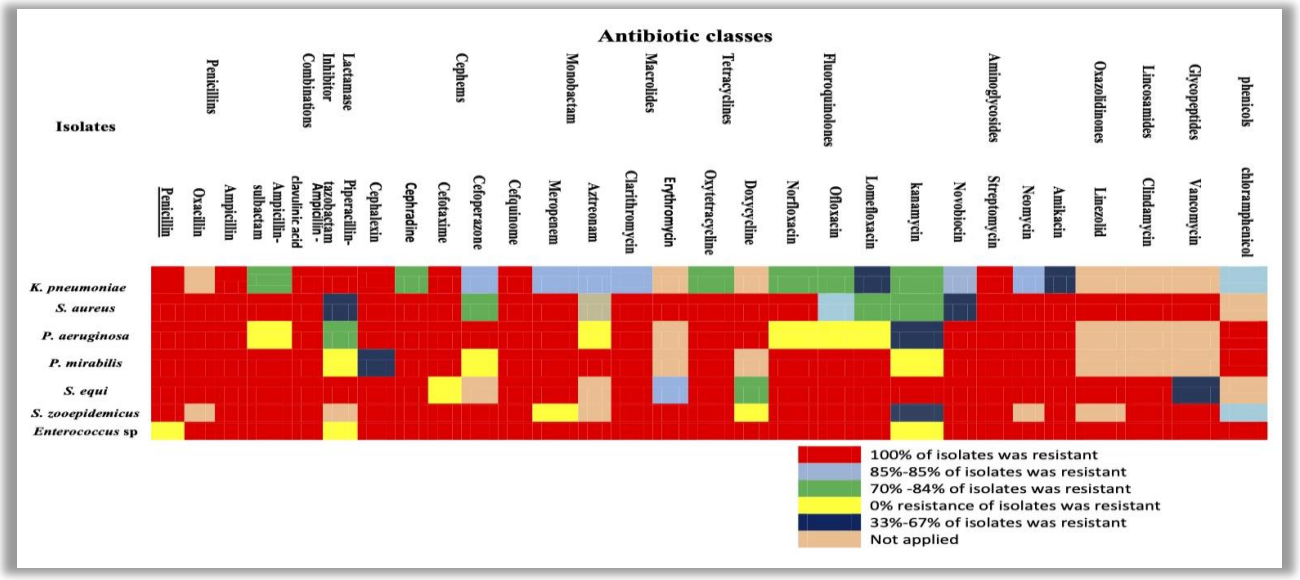

Figure 3. Heat map analysis showed the intensity of resistance of different isolates against different antimicrobial classes

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CONCLUSION

Stenotrophomonas maltophilia isolated from all organs, including the lung, is one of the first reports of isolation in Egypt. High rates of recorded antimicrobial resistance towards commonly used antibiotics emphasize the importance of individual bacteriological and antimicrobial susceptibility testing to guide antimicrobial therapy. The routine application of antimicrobials in the livestock industry has a dual effect, one acts as an advantage (beneficial for the health and productivity of the animal) while the other is considered as an important disadvantage with a global concern that is the significant evolution of different pathogenic bacterial strains having multidrug resistance (MDR) properties. In the present study, resistance monitoring data and risk assessment identified several direct and/or indirect predisposing factors to be potentially associated with MDR development in the equine health sector of Egypt. Affecting factors are inadequate veterinary healthcare, observing and controlling services, enhancing animal health knowledge among facility providers, and filling farmers' knowledge gap on drugs, and MDR which have resulted in the misuse and overuse of antibiotics leading to the evolution of antibiotic-resistant bacteria in equine in Egypt. Execution of extensive MDR, PDR, and XDR surveillance in equine and awareness programs for farmers along with the strengthening of the capacity of General Veterinary Services are recommended for effective containment of MDR emergence and spreading in the equine health sector in Egypt.

DECLARATION

Competing interests

Authors declare no conflict of interest.

Authors' contributions

Soumaya, S. A. El Shafii was responsible for project administration and validation. Nehal, M. Fawzy, 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. Kamelia, M. Osman, 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.

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