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CAIRO UNIVERSITY

Cairo university
Faculty of Veterinary Medicine

Detection of Multidrug Resistance Genes in Bacteria Causing Respiratory Disorders in Equine

A *Thesis Presented by*

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(M.V.M.Sc.., Fac. Vet. Med., Cairo University, 2015)

To
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(Bacteriology, Immunology and Mycology)

Under supervision of

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Abstract

Multidrug resistance is an issue of global concern. The current study aimed to detect the different pathogens causing respiratory disorders in the equine, describing the antimicrobial resistance profile with determining the types of drug resistance of each, investigating and analyzing the distribution pattern of the multi-drug resistance genes in the most predominant isolates. A total of 203 different samples were collected from 42 horse foals, 5 adult horses, and 4 donkey foals. All samples were subjected to bacteriological examination and the isolates were tested for different antibacterial agents. The findings indicated that 38 (74.5%) animals were positive for the bacterial isolation causing respiratory disorders. The most predominant isolates were *Klebsiella pneumoniae*. There's only 1 isolate of *Stenotrophomonas maltophilia,* is the first record in equine in Egypt. The antibiogram results' analysis revealed that, 73.6% of the isolates showed 100% resistance against several antibiotics. On the other hand, 59.1% of the isolates were pandrug resistant. Ten antibiotic resistance determinant genes coded on the chromosome of *K. pneumoniae* were investigated: Extended spectrum β-lactamases (ESBL) ($bla_{CTX\text{-}M}$, bla_{TEM}), metallo-beta-lactamases/ carbapenemases (bla_{VIM}, $bla_{NDM\text{-}1}$), carbapenemase (bla_{KPC}), Quinolones' target protective proteins "Qnr proteins" (*qnrB, qnrS*), aminoglycoside adenylyltransferase (*aadA1*), efflux pump (*AcrAB*) and porin protein mutation (*ompK35*). By analyzing the results, it was showed that the most predominant number of associated genes in a single strain was 5. Pareto chart elucidated that the association between ESBL, *AcrAB* and Qnr with the mutation of the porin protein was the most existed (26.7%). Interestingly, the sequencing results of the CTX-M PCR amplicons were typed as OXY-5 (50%), CTX-M-15 (40%) and CTX-M-27 (10%). The current study proved the indiscriminate use of antibiotics in the equine health sector of Egypt and the improper hygienic rules followed in the equine community. The represented results could be the first record of the MDR genes' predominance and their association among the *Klebsiella pneumoniae* strains and for the occurrence of the OXY-5 in Egypt. It may offer a helpful guide for scientists seeking new alternatives other-than antibiotics.

Keywords: Multidrug resistance genes, Extended spectrum β-lactamase producing-*Klebsiella pneumoniae*, CTX-M, OXY, equine, Egypt

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Contents

LIST OF FIGURES

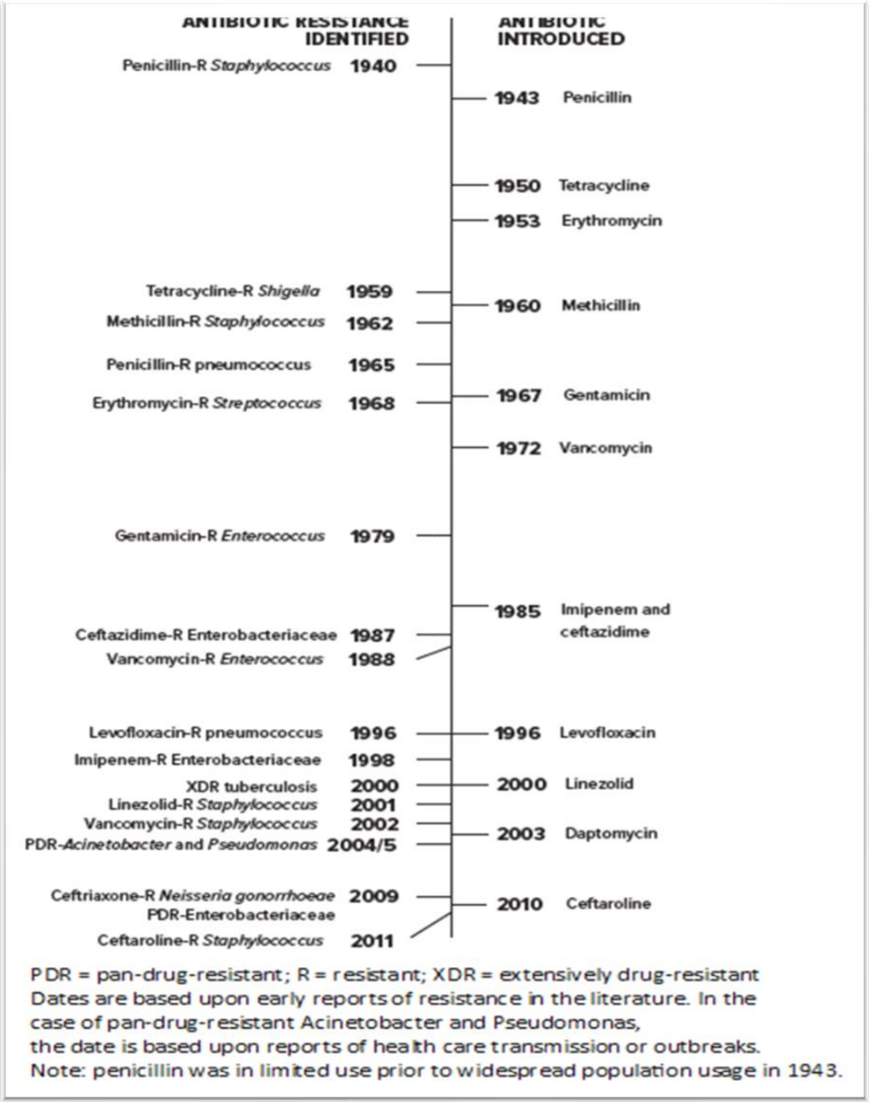
List of Abbreviations

CLSI	Clinical and Laboratory Standards Institute
MDR	Multidrug Resistance
AMR	Antimicrobial resistance
XDR	Extensively drug resistance
PDR	Pandrug resistance
Spp.	species
TMS	Trimethoprim-sulfamethoxazole
CIA	critically important antibiotics
BORSA	borderline-oxacillin-resistant *S. aureus*
MRSA	Methicillin resistant *S. aureus*
ESBL	Extended Spectrum Beta-Lactamases
ESBL-E	ESBL-*Escherichia coli*

Introduction

Multidrug resistance became a great and serious issue attacking the global health and so attracted the attention of most of the scientists worldwide. Most specialists (physicians, veterinarians, bacteriologists, etc……) are seeking for the causes and various alternatives for solving such a problem which threatening both human and animal health.

The first case of antibiotic resistance was recorded in 1940 for a case infected with a strain of *S. aureus* showing resistance against penicillin. During the period from 1950 to 1960 "the golden age of antibiotic discovery" did occur (illustrated in figure 1), during which approximately one-half of the widely used antibiotics today were discovered and was thought that the problem (antibiotic resistance) was solved. But unfortunately, new types of antibiotic resistance had emerged, and are still spreading dramatically in different bacterial genera and species (Ventola, 2015). Moreover, the cases in which a bacterial strain showed resistance against two or even all antibacterial agents in two or more antibacterial categories outstood as the more problematic issue. The expression "multidrug resistance" means the resistance of a bacterial strain against 2 or three antibiotic agents excluding the natural resistance of the bacterial species against certain antibiotic, for example the intrinsic natural resistance of the gram-negative species against most of the lipophilic antibiotics such as penicillin G, oxacillin, cloxacillin, nafcillin, macrolides, novobiocin, linezolid, and fusidic acid (Nikaido, 2009 and Ventola, 2015). Magiorakos *et al.,* 2012 defined the bacterial drug resistance and graded it into three categories according to the number of antimicrobial agents and classes to which the strain is resistant. These were defined according to the results of studying the resistance pattern of the most important bacteria and of public health significance which are *S. aureus, Acinetobacter spp., Enterococcus spp., P. aeruginosa, and Enterobacteriaceae* other than *Salmonella and Shigella.* The antimicrobial resistance results were guided by the clinical breakpoints provided by the Clinical and Laboratory standards (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST), and the US Food and Drug Administration (FDA). The three defined categories are: multidrug resistant (MDR), extensively drug resistant (XDR) and pandrug resistant (PDR). Nowadays, these nominates are also used in veterinary medicine, but calls asking for a support for applying studies on veterinary medicine pathogens (Ventola, 2015 and van Spijk, *et al*. 2019), indeed there are recent studies are carried out for approving them in veterinary medicine, but still very few (Sweeney, *et al*., 2018).



PDR = pan-drug-resistant; R = resistant; XDR = extensively drug-resistant
Dates are based upon early reports of resistance in the literature. In the case of pan-drug-resistant Acinetobacter and Pseudomonas, the date is based upon reports of health care transmission or outbreaks.
Note: penicillin was in limited use prior to widespread population usage in 1943.

Fig. (1): Developing Antibiotic Resistance: A Timeline of Key Events (ref.: Ventola, 2015)

Antimicrobial resistant-microbes (who're named "Superbugs") are found everywhere, people, animals, food, and the environment (in water, soil and air). They can spread between people and animals, and from person to person. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance (World Health Organization, 2016). Additionally, the extensive and misuse of antimicrobials either in veterinary medicine, human medicine or even agricultural field. Misuse and ineffective supervised practices of using antimicrobials and detergent in particularly veterinary medicine acts as one of the major causes of the antimicrobial resistance widespread, specially in the field the food animals' rearing, in which the animals act as reservoir of the antimicrobial resistant bacteria, transmitting them to human either through its food products (meat or milk sources) or via direct contact with human (Wu, *et al.*, 2019). Antimicrobial resistance is known to be widely spread specially in a population of horse; like that in hospitals or housing (stables) through the horizontal transmission of the resistance genes from bacteria to another or even a bacterial population from the infected animal and/or human to others (Barbosa and Levy 2000 and Van Spijk, *et al.*, 2019). The acquisition of the resistance genes is carried out via the transmission of the mobile genetic elements like the insertion sequences, transposons and integrons (Wu, *et al.*, 2019). There are various mechanisms of drug resistance recorded including: degradation of the drug by the action of an enzyme, alteration of the target site of the drug, alteration of the membrane permeability for the drug and drug efflux pump (Ogawa, *et al.*, 2005).

WHO considered AMR as a global health concern for several reasons (World Health Organization, 2020):

1- The emergence and spreading of multidrug resistant and pandrug resistant bacteria (superbug) globally, resulting in untreatable infections, although the availability of variant antibiotics
2- There's no new antibiotics introduced as a tool of facing AMR, in addition to the lack of presence of highly qualified antibiotics.
3- Untreatable and difficultly treated infection due to the continually arising and spreading of antibiotic resistant pathogens. Urgently needed antibacterial for the gram negative carbapenemase-

producing bacteria, but even if this is achieved, still the abuse of the antibiotics is considered as an impediment.

4- The cost effect of the AMR on the national economies of either the patient (decrease the productivity and his income), healthcare foundations (prolonged stay of patient in the hospital and intensive care) and so the whole country.
5- Critical surgeries including caesarian sections, cancer excisions and organs' transplantation became more critical for the wide spread of AMR pathogens with inefficient hygienic measures.

Actually, there are several drivers of AMR including: abuse of the antibiotics, lack or insufficiency of the hygienic measures and sanitation either for human or animals, difficult access to clean water, poor healthcare and veterinary services for both human and animals respectively, with inefficient implementation of the infection prevention and control rules, deficient quality medicines and vaccines, lack of awareness and finally and lack of commitment to legal laws (World Health Organization, 2020). Indeed, there are various sectors and stakeholders have to communicate and cooperate for overcoming this great issue such as those incorporated in the sectors of healthcare, veterinary medicine (both for animal and aquatic health), terrestrial and plant health, food and feed production and the environment sector. These different sectors have to work together to design a control system including programmes, plans, legalizations and research projects for the improvement of the global health and minimize the adverse effects of AMR. Also, we need to encourage the investment in the research and production of new antimicrobials, vaccines and improvement of the diagnostic tools targeting the critical bacteria like that the gram-negative bacteria (carbapenem-resistant Enterobacteriaceae). Several countries initiate a project for facing the problem including: Germany, United States of America, Sweden, and the United Kingdom. They set a reimbursement model and committed to the framework of the Global Action Plan (GAP) on AMR set out by the World Health Organization 2015. Additionally, three foundations collaborated to engage all the stakeholders to the GAP, these foundations are: FAO (Food and Agriculture Organization of the United Nations), OIE (World Organization of the Animal Health) and WHO (World Health Organization) (World Health Organization, 2020).

Respiratory infections in equine have economic significance for the horse owner/manager in many ways as it causes (Wood, *et al.*, 2005 and Giedt, 2014):

- Losing of training time.
- Damage to the respiratory tract can take weeks for full repair and return to work.
- Competition horses may miss valuable point shows or competitions.
- The expense of treatment includes both medications and time spent by the owner.
- Concern about spread of disease through a facility can impact boarding and training revenues

Certainly, studies on the multidrug resistance in the field of equine medicine are considered few, mostly approaching Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Extended spectrum B-lactamases (ESBL)-producing *E. coli* (Anderson *et al*. 2009; Van Duijkeren *et al.* 2010; Maddox *et al.* 2012; Dierikx *et al.* 2012 and Walther *et al.* 2014) and is almost null in Egypt.

In the current study, we focused on the detection of the drug resistance determinant genes expressing beta-lactamases production (TEM and CTX-M), metallo-beta-lactamases/carbapenemases (VIM, NDA, KPC), aminoglycosides transferases, quinolones targets' protective proteins, efflux pump and porin protein mutation/deletion genes in the most predominant bacterial isolates causing respiratory infections.

The protocol of the work is:

- Isolation and identification of different bacteria from equine showing respiratory disorders, or showed respiratory disorders before death.
- Antimicrobial sensitivity test for the isolates against different antimicrobial agents.
- Detection of multidrug resistance genes in the most predominant isolates.
- Statistical analysis of the results.

Review of Literature

I- Economic importance of equine industry

Equine including donkeys, mules, ponies and horses (working animals) still are the power engine in rural specially in developing countries. Although horses are non-food production animals (Valette. 2015).

Horse industry is considered one of the most important part of the United States' economies, involving agriculture, business, sport, gaming, entertainment and recreation (Jaffe and Hermonot, 2016). The horse industry has a direct economic effect on the U.S. of $39 billion annually, (paying $1.9 billion in taxes to all levels of government) and provides 460,000 full-time equivalent jobs (Department of Agricultural Leadership. 2017). Many activities are involved under the definition of "Equine Industry" such as: all activities involved in breeding, raising, selling and using horses for commercial and recreational purposes. In addition to, many stakeholders are involved in such industry like, pleasure horse owners, racing horse owners, working horse owners, personals working in the boarding facilities, rehabilitation facilities, therapeutic riding facilities, vets, farriers, racetracks, showing events and riders who do not own horses but are involved in the industry (Jaffe and Hermonot, 2016).

The government of Egypt founded the Royal Agricultural Society (RAS) in 1908, which is known today as the Egyptian Agricultural Organization (EAO). The E.A.O. plays a very important role in caring,