

NARRATIVE REVIEW

Review of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* as venereal pathogens in horses

Melanie Scholtz¹  | Alan John Guthrie¹  | Richard Newton² |
Martin Lance Schulman³ 

¹Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

²Department of Veterinary Medicine, University of Cambridge, Cambridge, UK

³Section of Reproduction, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa

Correspondence

Melanie Scholtz, Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa.
Email: mel.scholtz@gmail.com

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Abstract

Three bacteria extensively acknowledged as venereal pathogens with the potential to induce endometritis include *Taylorella equigenitalis*, the causative agent of contagious equine metritis (CEM), specific strains of *Pseudomonas aeruginosa*, and certain capsule types of *Klebsiella pneumoniae*. The United Kingdom's Horserace Betting Levy Board recommends pre-breeding screening for these bacteria in their International Codes of Practice and >20 000 samples are tested per annum in the United Kingdom alone. While the pathogenesis and regulatory importance of CEM are well established, an evaluation of the literature pertaining to venereal transmission of *P. aeruginosa* and *K. pneumoniae* was lacking. The aim of this review was to evaluate published literature and determine the significance of *P. aeruginosa* and *K. pneumoniae* as venereal pathogens in horses. Literature definitively demonstrating venereal transmission was not available. Instead, application of molecular typing methods suggested that common environmental sources of contamination, such as water, or fomites be considered as modes of transmission. The presence of organisms with pathogenic potential on a horse's external genitalia did not predict venereal transmission with resultant endometritis and reduced fertility. These findings may prompt further investigation using molecular technologies to confirm or exclude venereal spread and investigation of alternative mechanisms of transmission are indicated.

KEYWORDS

horse, *Klebsiella pneumoniae*, pre-breeding screening, *Pseudomonas aeruginosa*, venereal

1 | INTRODUCTION

Venereally transmissible bacterial pathogens, while not the most common cause of endometritis,^{1–4} contribute to the estimated prevalence of 10%–15% of persistent breeding-induced endometritis (PBIE) reported in intensively managed Thoroughbred mares.⁵ Endometritis results in lowered reproductive performance,^{6–8} with consequent

significant economic and genetic losses.^{9,10} Further economic losses may result due to restrictions on international trade and movement of horses and semen and embryo shipments, as in the case of contagious equine metritis (CEM).¹¹

The three bacteria most widely considered as transmissible venereal pathogens capable of causing endometritis are *Taylorella equigenitalis*, the causative organism of CEM, certain strains of *Pseudomonas*

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aeruginosa and *Klebsiella pneumoniae* with certain capsule types. Outbreaks of endometritis associated with these three bacterial species have been reported.^{12–19} These bacteria are all similarly harboured as colonists of the epithelia in smegma associated with predilection sites within the external genitalia, producing a subclinical carrier state which is challenging to detect.^{13,17,20–25} These sites are the clitoral fossa and sinuses of mares and the urethra, urethral fossa and preputial *lamina interna* of stallions.^{13,20–24,26}

This insidious pathogenesis and the undoubted dramatic impacts on reproductive performance of the initially-reported CEM outbreaks in the late 1970s^{19,27,28} swiftly prompted several national Thoroughbred authorities to develop various ‘stud health’ programmes and ‘codes of practice’. These measures aimed to detect affected horses, limit their impact at the population level, and improve reproductive performance. Contagious equine metritis is a World Organisation for Animal Health (WOAH) listed disease²⁹ and consequently a controlled and, or notifiable disease in several countries, including but not limited to Australia, Japan, South Africa, the United Kingdom and the United States.^{30–34} Although neither *P. aeruginosa* nor *K. pneumoniae* are notifiable by law in these countries, they are regarded by certain regulatory authorities as important venereal pathogens that warrant pre-breeding screening of stallions and mares to control venereal disease.^{35–37} For example, pre-breeding testing for *T. equigenitalis*, *P. aeruginosa* and *K. pneumoniae* is advocated by the voluntary Codes of Practice of the UK’s Horserace Betting Levy Board (HBLB)³⁵ and >20 000 samples were tested per annum in the United Kingdom from 2016 to 2023.³⁸ These samples comprise reproductive tract samples, including those submitted for pre-breeding screening, pre-export testing, and those obtained from horses with clinical disease. Multiple samples may originate from a single horse. These Codes have more recently been branded as ‘international’ in light of them being adopted by multiple countries, including France, Germany, Ireland and Italy. Pre-breeding screening for these above-mentioned bacteria is conducted by sampling genital predilection sites in mares and stallions and subjecting samples to either bacterial culture or PCR assays to confirm or rule out the subclinical carrier state.^{21,35,39} Surprisingly, however, there is a paucity of reports linking both *P. aeruginosa* and *K. pneumoniae*, when obtained from samples derived from genital swabs, with either venereal transfer or disease occurrence. Managemental inputs and costs associated with testing are significant when viewed collectively and it is therefore imperative that such costs are substantiated by appropriate evidence. Recently, a focused review examined the evidence concerning venereal transmission of *P. aeruginosa* and emphasised the necessity for comprehensive assessments of both *P. aeruginosa* and *K. pneumoniae* as potential venereal pathogens.⁴⁰ Contagious equine metritis is extensively reported, with a well-established pathogenesis, clinical and regulatory importance^{11,16,17,23,24,27,41–48} and as such it will not be reviewed here. This review will interrogate the literature reporting *P. aeruginosa* and *K. pneumoniae* as venereal pathogens.

2 | PERSISTENT BREEDING-INDUCED ENDOMETRITIS

The mare’s cervix relaxes during oestrus, allowing for direct intrauterine deposition of semen by the stallion at ejaculation. This results in the mare’s endometrium being challenged by the intra-uterine transfer of semen as well as bacteria found on the external genitalia of both the mare and stallion and endometritis may result.^{49,50} All mares may be said to develop breeding-induced endometritis. This is typically short-lived and transient with most mares resolving the post-breeding uterine contamination and associated inflammation without intervention within 24–36 h.^{51,52} If the inflammation is not resolved, persistent breeding-induced endometritis (PBIE) occurs.⁵ Importantly, up to 15% of Thoroughbred mares may develop PBIE and require treatment.^{5,6}

Bacterial endometritis is often not apparent and only evidenced by a failure to conceive, early embryonic death (EED) or a shortened luteal phase in affected mares. The consequence is subfertility or infertility arising from either conception or early gestational failure.^{6,8,52–54} Persistence of infection in mares is affected by age, parity, barren years and uterine biopsy grade, with maiden mares being the most resistant to infection.^{7,55–58}

3 | KEY CONCEPTS: MARE REPRODUCTIVE CATEGORISATION AND VENEREAL PATHOGENS

Typically, maiden and multiparous mares without evidence of previous foaling injuries or other abnormalities associated with disrupted normal genital anatomy, function or conformation are able to resolve the ensuing acute post-breeding endometritis caused by opportunistic bacteria and are termed ‘resistant mares’. Mares with genital anatomical, functional or conformational abnormalities, myometrial abnormalities or injuries of the reproductive tract are unable to effectively resolve the ensuing post-breeding acute endometritis and are termed ‘susceptible mares’.^{49,53,54,56,58–67} Opportunistic bacteria are able to cause clinical endometritis in mares with compromised uterine defences^{53,55,61,68–70} but do not produce epidemic disease and are therefore not considered venereal pathogens.⁶⁹ Venereal bacterial pathogens differ from opportunistic bacteria in that they are considered capable of causing acute endometritis, not only in susceptible mares, but also in resistant mares and may result in outbreaks of post-mating endometritis due to being transmitted between stallions and mares in either direction.^{13,15,18,69,71}

4 | SEARCH METHODOLOGY

Systematic searches of electronic databases were performed for *P. aeruginosa* and for *K. pneumoniae* on 7 May 2023 and updated on 14 June 2023. Our search strategy is provided in Table S1.

Publications addressing venereal transmission and or genetic relatedness of *P. aeruginosa* or *K. pneumoniae* in genital swab samples or in semen were included, as were reports of outbreaks of endometritis caused by either *P. aeruginosa* or *K. pneumoniae*. Studies reporting only the prevalence of these bacteria in genital swabs, endometrial, or semen samples but not addressing venereal transmission or genetic relatedness have not been discussed below (Figure S1 and Text S1).⁷² The lack of uniformity in study design, study populations, sampling sites and diagnostic methods employed precluded the possibility of conducting a meta-analysis. A summary of the studies included in this review are provided in Tables S2 and S3.

5 | PSEUDOMONAS AERUGINOSA

5.1 | Transmission by carrier stallions

Isolation of *P. aeruginosa* from the genital tract of mares was first described by Hughes et al. in 1966⁶³ and the bacterium was subsequently described as an equine reproductive tract pathogen in 1967⁷³ and in 1975.⁷⁴ Furthermore, it has been listed as a venereal bacterial pathogen in various texts.^{69,75–77}

To document effects of *P. aeruginosa* on fertility, Hughes et al.⁶³ bred nearly 800 maiden, barren and foaling mares to *P. aeruginosa* carrier-stallions. Mares were tested by pre-breeding cervical and uterine cultures and those with negative results were bred without treatment. Those with clinical signs of infection and positive cultures were treated with intrauterine antibiotics prior to breeding. Subsequent to covering, *P. aeruginosa* was recovered from the cervixes or uteri in approximately 10% of mares. In the view of Hughes et al.,⁶³ this established the occurrence of venereal transmission. It was however, also suggested that obtaining a positive *P. aeruginosa* culture from the mare's reproductive tract in the absence of clinical signs of inflammation, infection or pneumovagina, should be considered incidental as *P. aeruginosa* was recovered from the anterior genital tract in healthy mares that had not been bred.⁶³ Further observations were limited to 70 infected mares for which breeding and clinical records were available. Post-breeding, maiden mares readily eliminated *P. aeruginosa*, whereas foaling and barren mares appeared more susceptible to establishment of *P. aeruginosa* in their reproductive tracts, with resultant endometritis and subfertility. It was further noted that breeding a susceptible mare to a *P. aeruginosa* carrier-stallion with establishment of a *P. aeruginosa* infection might prevent conception. Interestingly, nearly 50% of mares treated with antibiotics prior to breeding were positive on post-breeding bacterial culture, which, arguably, may indicate that antimicrobial treatment reduced numbers of commensal bacteria, allowing the more resistant *P. aeruginosa* to proliferate. Notably, mares were sampled from the cervix and uterus and not the clitoral predilection sites. Thus, it is uncertain whether mares were inapparent carriers of *P. aeruginosa* prior to breeding. Furthermore, common environmental sources of *P. aeruginosa*, such as contaminated water, were not excluded from consideration.⁷⁸ Testing for *P. aeruginosa* was solely by bacterial culture without application of typing

methods and prevented confirmation that the organism isolated from both stallion and mare were of the same type. While venereal transmission was reported, it was also emphasised that mares are exposed to many potential pathogens at the time of breeding and that resistant mares were less likely to be affected by this exposure than susceptible mares.⁶³

In 1967, Hughes et al.⁷³ reported that conception rates of 25 stallions harbouring *P. aeruginosa* (isolated from urethral swabs or semen samples) ranged from poor to good, with approximately 25% having conception rates of 71% or higher. Venereal transmission of *P. aeruginosa* with resultant endometritis thus cannot be assumed to occur in all instances. Furthermore, consideration of the role of mare status (susceptible or resistant) was lacking in determining whether exposure to potential pathogens during coitus resulted in infection and the extent to which this status may affect mare fertility, irrespective of *P. aeruginosa* exposure. It was suggested that stallions from which *P. aeruginosa* was recovered should not be considered unfit for breeding but that these stallions should be viewed as a potential source of contamination to susceptible mares. This emphasised the benefit of having resistant mares in a breeding programme. This is frequently not the case in Thoroughbred breeding systems, however, with many of the high value mares retained in breeding programmes being multiparous and older and thus more likely to be classified as susceptible.⁷⁹

Contradictory observations were reported in 1975, in two stallions with consistent isolation of *P. aeruginosa* from their semen.⁵⁶ Most multiparous mares bred to the first stallion developed clinical infections from which *P. aeruginosa* was isolated, whereas all maiden mares conceived. These observations suggested that stallions which harbour *P. aeruginosa* have poor conceptions rates when bred to multiparous mares and supported previous works demonstrating maiden mares were at lower risk of developing mating induced infections.^{63,73} The second stallion bred a larger number of mares, with over 78% confirmed as pregnant and 67% produced live foals. This suggested that stallions harbouring *P. aeruginosa* may have acceptable pregnancy rates, comparable to the 53%–100% cumulative pregnancy rates recorded for Thoroughbred stallions in the United Kingdom.^{80,81} However, the mares were of unknown reproductive status and age and these factors may individually and cumulatively have profoundly affected both conception and foaling rates.^{7,80,82–89} Furthermore, the first stallion covered fewer mares, which limited valid comparison of the conception rates of these stallions.⁵⁶

A stallion, diagnosed with seminal vesiculitis due to *P. aeruginosa*, was bred to mares by artificial insemination (AI) with raw or polymyxin-B sulfate-extended semen.⁹⁰ First cycle pregnancy rates were 10% for mares bred with raw semen, compared with 78% for those bred with extended semen.⁹⁰ Interestingly, none of the mares that returned to oestrus post-AI showed isolates of *P. aeruginosa* recovered from culture of uterine swabs or biopsies. Unfortunately, mare age and reproductive status were unavailable and their effects on differences reported between the two groups of mares is unknown. This stallion had an internal infection with leukospermia

that consistently yielded a heavy growth of *P. aeruginosa*. Consequently, these findings should be extrapolated with caution to the more commonly reported scenario of transient colonisation of the external genitalia.⁹¹

A more recent case study attempted to determine whether *P. aeruginosa* harboured on a stallion's external genitalia would be transmitted during natural service or AI.⁹² Mares were confirmed negative for *P. aeruginosa* prior to breeding by bacterial culture of samples collected from the clitoral fossa, vaginal vestibule and endometrium. While the report lacked clarity, the authors seemingly stated that approximately 50% of mares bred by natural service were positive for *P. aeruginosa* post-breeding and thus concluded that venereal transmission had occurred.⁹² Owing to the confusing nature of the report, the results were challenging to interpret. Furthermore, definitive demonstration of venereal transmission using molecular methods was lacking.

5.2 | Typing of genital isolates

Typing of *P. aeruginosa* strains plays an important role in elucidating routes of transmission of these bacteria amongst horses. Prior to 2009, the only reported typing of genital *P. aeruginosa* strains in horses was in 1982 by Atherton and Pitt¹³ with serological typing, phage typing and haemagglutination techniques used to type bacterial isolates and in 1993 by Kenney et al.⁹³ who used serotyping. An overriding conclusion of the 1982 report was that correlation between strain type and pathogenicity could not be established, and consequently all strains of *P. aeruginosa* should be considered potentially pathogenic.¹³ An additional description of three outbreaks seemingly supported the assumption of venereal transmission.¹³ One stallion and six mares with clinical signs of endometritis were involved in the first outbreak. *Pseudomonas aeruginosa* type O3, H3 phage type A was isolated from cervical swabs from all mares and the same strain was recovered from the penis of the stallion that bred them. One stallion and four mares without clinical endometritis signs were involved in the second outbreak. *Pseudomonas aeruginosa* type O3, H3 phage type A was isolated from clitoral swab samples and the penis of the stallion. The *P. aeruginosa* types isolated from the first and second outbreaks were indistinguishable, with no obvious links between them. The third outbreak involved one stallion and nine mares without clinical endometritis. *Pseudomonas aeruginosa* was recovered from the stallion, the cervix of five mares, and clitoris of four mares. Isolates were type O9, H5 with identical phage patterns.¹³ The belief in the significance of any genital isolate of *P. aeruginosa* for venereal transmission has endured over time, as evidenced by the HBLB International Codes of Practice.³⁵ This persistence remains despite the report being limited by the diagnostic and strain typing methods available at the time,⁷⁸ which consequently could not definitively demonstrate venereal transmission based on authoritative genotyped transmission data. Furthermore, the report did not specify epidemiological links between horses, farms or outbreaks, and a common environmental source of contamination or fomite transmission in the first and second outbreak cannot be ruled out.^{12,78} A later observation from non peer-

reviewed conference proceedings that serotypes of *P. aeruginosa* isolated from mare uteri, in most instances, differed to those isolated from stallions, was inconsistent with venereal transmission.⁹³ These two opposing findings, using different methodologies, highlight the need for studies applying currently available molecular technology, preferably to the level of discrimination of the whole genome sequence.

However, some molecular typing methods have been key to confirming routes of transmission, occurrence of venereal spread and possibly, variations in strain pathogenicity.^{12,78,94} In human medicine, various molecular typing techniques have revealed the epidemiology of *P. aeruginosa* populations.⁹⁵ These techniques include multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE), random amplified polymorphic DNA analysis, repetitive extralpalindromic PCR analysis and restriction fragment length polymorphic DNA analysis.⁹⁵ Additionally, the use of genome sequencing has elucidated characteristics of *P. aeruginosa* associated with pathogenicity and identified strains considered non-pathogenic to humans.⁹⁴ There are limited reports of the application of such molecular techniques to populations of *P. aeruginosa* in horses, with Tazumi et al.,⁹⁶ Kidd et al.⁷⁸ and Allen et al.¹² reporting on the genetic relatedness of equine *P. aeruginosa* isolates.

In 2009, Tazumi et al.⁹⁶ examined the genetic relatedness of 63 *P. aeruginosa* isolates recovered from 63 horses in Ireland over a 5-year period using an enterobacterial repetitive intergenic consensus (ERIC) random amplification of polymorphic DNA (RAPD) PCR. Swabs were originally collected from various sites, including the urogenital tract and from semen. Twenty-four distinct genotypes were obtained, thus revealing substantial clonal heterogeneity, although certain genotypes appeared to persist and recur within this population. The authors reported good repeatability of results when examining isolates with ERIC2.⁹⁶ Unfortunately, it was not clear how many isolates originated from the urogenital tract or from semen, nor was it stated whether an association between genotypic clustering and sampling site existed. Furthermore, information regarding whether horses were epidemiologically linked was not provided,⁷⁸ thus no inference could be made regarding the genetic relatedness of *P. aeruginosa* isolates that originated from the urogenital tract and semen samples based on the report of Tazumi et al.⁹⁶ Similarly, in a more recent analysis, no inference could be drawn regarding the genetic relatedness of equine genital isolates. Amongst the 41 isolates from various species subjected to whole-genome sequencing (WGS), 15 originated from equine genital sources. Unfortunately, information regarding the genetic relatedness or epidemiological links of the sampled horses was not available. Overall, the strains exhibited considerable diversity.⁹⁷

Kidd et al.⁷⁸ examined samples collected from 2040 mares and 18 stallions from 66 stud farms in south-east Queensland, from 2005 to 2009, to determine the relatedness of genital *P. aeruginosa* isolates. Mare samples were either clitoral or uterine swabs obtained from those with suspected infection or for routine screening and stallion samples were collected from the urethral fossa. Isolates of *P. aeruginosa* obtained on bacterial culture of samples further underwent qPCR, ERIC-PCR strain typing and antimicrobial susceptibility testing. Of the 2040 mares tested, 93 (4.6%) were positive for

P. aeruginosa while none of the 18 stallions were positive.⁷⁸ The observation that no stallions tested positive may suggest that transmission by coitus did not play a major role, that sample technique was inadequate or that stallion management practices prevented colonisation of the external genitalia.⁷⁸ The genital tracts of Thoroughbred mares from that region were found to harbour a diverse range of unrelated *P. aeruginosa* organisms, but 53/93 (57%) mares did share clonal complexes of *P. aeruginosa*. It was suggested that transmission of the organism via fomites or a common environmental source should be considered or alternatively, that some *P. aeruginosa* strains may have a tropism for the equine reproductive tract.⁷⁸ These findings were similar to those of Tazumi et al.⁹⁶ in that both found a high degree of clonal heterogeneity with some small clusters of shared clonal complexes, but dissimilar in that Kidd et al.⁷⁸ reported a single dominant genotype.⁷⁸ This may be because Tazumi et al.⁹⁶ examined samples collected from various sites, whereas Kidd et al.⁷⁸ limited sample collection to the reproductive tract.

In 2011, Allen et al.¹² assessed the usefulness of PFGE in determining venereal transmission of *P. aeruginosa*. Investigation of an outbreak of endometritis revealed that the same PFGE strain of *P. aeruginosa* was isolated from the external genitalia of three stallions and the uteri of four mares. However, as these three stallions had bred different mares, venereal transmission could not account for all instances of spread of the organism, and thus a common environmental source of contamination was suspected. These findings contradicted assertions by Atherton and Pitt,¹³ that multiple outbreaks of *P. aeruginosa* endometritis occurred due to venereal transmission. Interestingly, transmission of *P. aeruginosa* to all mares served by affected stallions did not occur and there were no subsequent infections in mares following the implementation of a new water source for cleaning.¹²

In alignment with the proposals by Kidd et al.⁷⁸ and Allen et al.,¹² to consider a shared environmental source of *P. aeruginosa* contamination, a recent investigation of *P. aeruginosa* sequence types (STs) from mares with endometritis and their corresponding drinking water reported instances of identical STs in both sources. This implied the potential for bidirectional contamination between mares and water supplies, underscoring the need to consider water's role in mare hygiene, particularly before breeding procedures.⁹⁸

In summary, contradicting the arguably circumstantial evidence in earlier reports,^{56,63,73,92} more recent findings based on typing isolates of *P. aeruginosa*^{12,78,93,98} suggested that venereal transmission may not be the main mechanism of transmission of related *P. aeruginosa* genital isolates amongst horses. Rather, fomites or common environmental sources should be considered. While Atherton and Pitt¹³ described outbreaks of similar strain types in mares and stallions, they were limited by the lack of molecular technology, as employed in later studies.^{12,78} Thus, although *P. aeruginosa* is often referred to as a venereal pathogen, substantive evidence to this effect appears to be limited.^{12,78,93} The lack of molecular methodologies in earlier years, limitations associated with conventional bacterial isolation and typing, and limited characterisation of strains involved in endometritis, might have resulted in the assumption that venereal transmission had occurred for a solitary strain of *P. aeruginosa*.

6 | *KLEBSIELLA PNEUMONIAE*

6.1 | Transmission by carrier stallions

Venereal transmission of *K. pneumoniae* was first reported in 1972 when 10 of 32 mares became infected after being bred to a persistently infected stallion.¹⁴ Those infected consisted of foaling ($n = 4$), maiden ($n = 1$) and barren ($n = 5$) mares. This result suggested that both potentially resistant and susceptible mares were affected. The *K. pneumoniae* isolated from the stallion and all infected mares had identical colony morphology, capsule type and antibiotic sensitivity and resistance. Consequently, it was concluded that venereal transmission had taken place. This report was widely cited to support the supposition that *K. pneumoniae* is a venereal pathogen.^{18,56,69,99–101} Of the 10 infected mares, only the five barren mares were examined bacteriologically prior to breeding and found to be negative. While not explicit, it appeared that only cervical swabs were obtained, thus not excluding an inapparent carrier status in the mares. This report differed from others in that the stallion was shown to be persistently infected. Both topical and parenteral antimicrobial treatment were ineffective in eliminating the organism that colonised the stallion's external genitalia.¹⁴ In this case, *K. pneumoniae* was isolated as far proximal as the renal pelvis and bladder on post-mortem examination. This implied establishment of a virulent strain in the genitourinary tract of the stallion and not transient contamination or colonisation of the external genitalia which is suggested as the more common scenario.^{18,55,91}

6.2 | *Klebsiella pneumoniae* capsule types implicated in outbreaks of endometritis

It has frequently been stated that *K. pneumoniae* capsule types 1, 2 and 5 are capable of venereal transmission, whereas other capsule types may be part of the normal flora of equine external genitalia.^{14,18,35,69,102} Capsule types 1 and 5 were those most commonly associated with outbreaks of *K. pneumoniae* endometritis in Newmarket between 1967 and 1975.¹⁸ In 1972, however, capsule types 2 and 5 were reportedly isolated from all samples obtained from outbreaks of endometritis and submitted to the Equine Research Station, Newmarket, UK.¹⁴ Outbreaks associated with capsule type 2 were previously reported by the Equine Research Station in 1963 and 1964.¹⁸ During the period 1967 to 1975, stallions that served mares with confirmed *K. pneumoniae* infection were sampled via preputial swabs and capsule type 7 was most frequently isolated. Capsule type 7 was considered as a possible component of the normal genital flora as it did not appear to be associated with uterine infections in mares.¹⁸ In one instance, a stallion was apparently temporarily infected with *K. pneumoniae* capsule type 5 after serving a subclinically infected mare and it was suggested that the stallion transmitted this infection to another mare via the venereal route.¹⁸ It was concluded that venereal transmission had occurred based solely on serotyping to determine capsule type. Considering the above, it would seem reasonable to conclude that capsule types 1, 2 and 5 appeared to be pathogenic in terms of their ability to cause uterine infections. It is similarly

reasonable, however, to conclude that venereal transmission was not proven, as serotyping was used in all instances to determine capsule type and this method is associated with several limitations, including extensive cross reactivity amongst certain serotypes and subjective interpretation of results.^{101,103-105} Furthermore, fomites or a common environmental source of contamination were not considered.

In a Japanese survey of cervical, faecal and nasal swabs obtained from mares and genital swabs from stallions, it was found that *K. pneumoniae* capsule type 1 predominated in cases of endometritis.⁹⁹ Capsule types 1 and 7 and those that were un-typable were isolated from stallions. As only capsule type 1 was implicated in endometritis and capsule type 7 was not, these findings supported those of Platt et al.¹⁸ that postulated that capsule type 7 was part of the normal genital flora and not typically associated with bacterial endometritis. The Japanese survey⁹⁹ presumed that venereal transmission had occurred, as only mares bred to stallions carrying heavily encapsulated capsule type 1 developed endometritis, whereas those bred to stallions carrying less heavily encapsulated capsule type 1 did not. It was further presumed that the increase in *K. pneumoniae* isolation from stallions for 2 years after the increase in *K. pneumoniae* endometritis cases was due to venereal spread of the pathogen. Venereal transmission, however, was not definitively demonstrated, as genotyping was unavailable, and common environmental sources or fomites were not investigated. Furthermore, clitoral swabs were not obtained prior to mating to determine whether mares were carriers of heavily encapsulated capsule type 1. Interestingly, the number of reported *K. pneumoniae* endometritis cases had increased concurrently and in the same area as an outbreak of CEM.⁹⁹ Speculatively, the use of antibiotics in the treatment of CEM may have altered the genital flora, favouring the growth of more resistant organisms such as *K. pneumoniae*, resulting in the observed increase in its isolation from mares and stallions. Similar observations of such disruption were made during the more recent South African CEM outbreak.¹⁰⁶ Later, the presence of similar plasmid profiles in *K. pneumoniae* capsule type 1 isolated from infected mares and from stallions seemingly supported the concept of venereal transmission.¹⁰⁷ In contrast, plasmid profiles of capsule type 7 isolated from mares and stallions were varied.¹⁰⁷ It has, however, been demonstrated that identical plasmids can be disseminated amongst diverse bacterial strains of *K. pneumoniae* via horizontal gene transfer.¹⁰⁸ Thus, distantly related bacteria may share certain plasmids. A greater degree of certainty regarding genetic relatedness should be attained through MLST or other more discriminatory molecular techniques such as WGS.

In summary, although various sources referenced this bacterium as a venereal pathogen,³⁵ literature definitively demonstrating venereal transmission of *K. pneumoniae* is not yet available, and information regarding phylogenetic proximity of strains isolated from equine genital tracts is limited.

7 | IMPLICATIONS FOR EVIDENCED-BASED BREEDING MANAGEMENT

In summary, mares and stallions may harbour potentially pathogenic bacteria in the clitoral fossa or sinuses of mares, and on the penis or in

the urethral fossa and urethra of stallions, and these may be introduced into the mare's uterus during breeding, whether by natural or artificial means.^{13,35,55,56,91,109-112} Although not definitively demonstrated, several studies have suggested that despite the presence of potential pathogens on the external genitalia of stallions and the associated potential for their coital transmission, the majority of mares did not become infected and, or, pregnancy rates were unaffected.^{55,56,99,111} Notably, mares bred to stallions positive for potential pathogens on the external genitalia did not exhibit an elevated frequency of positive post-breeding uterine cultures, and bacteria isolated from these uteri often differed from those isolated from stallions.⁵⁵ Whether or not this demonstrated a lack of venereal transmission or more simply the mares' ability to clear the organisms from their reproductive tracts post-breeding cannot be inferred, especially as information on mare parity and classification as resistant or susceptible was typically not provided. It may therefore be concluded that during natural mating the presence of organisms with pathogenic potential on stallions' external genitalia does not predict venereal transmission with resultant endometritis and reduced fertility. Furthermore, some stallions carrying *P. aeruginosa* or *K. pneumoniae* on their genitalia achieved acceptable pregnancy rates, and not all mares exposed to these pathogens during the course of being bred subsequently became infected.^{12,14,55,63,73,99,111} Resistant mares have demonstrated the ability to conceive even when challenged with these pathogens on a stallion's genitalia.^{56,63} The detection of either of these bacteria from genital swabs thus provides insufficient evidence of the presence of venereally transmissible pathogenic bacteria capable of causing disease. Interestingly, a recent brief review of venereal transmission of *P. aeruginosa*, suggested this organism should be categorised as an opportunistic, not venereal, pathogen and managed similarly to β -haemolytic *Streptococci* and *E. coli*, which are considered part of the normal microflora of a horse's genitalia and may be mechanically transmitted during coitus.⁴⁰

8 | FUTURE DIRECTIONS

The limited evidence supporting venereal transmission of *P. aeruginosa* or *K. pneumoniae*, coupled with the observational nature of studies, highlight the need for contemporary investigations utilising advanced molecular technologies to definitively confirm or dismiss venereal transmission. In this regard, experimental studies employing molecular techniques such as WGS are imperative to elucidate the transmission dynamics, encompassing both venereal and fomite-mediated routes, as well as potential environmental sources of contamination. Recent findings from France, employing a genomic approach, revealed that hypervirulent and, or multidrug-resistant (MDR) strains of *K. pneumoniae* were undetected by a PCR assay targeting capsule types 1, 2, and 5 in equine genital swabs collected for pre-breeding screening,¹¹³ suggesting the assay may not be comprehensive enough to detect all potentially pathogenic types of *K. pneumoniae*. These findings underscore the necessity of employing

modern techniques. Future research and surveillance may wish to consider the detection and transmission of MDR strains of *P. aeruginosa* and *K. pneumoniae*, along with hypervirulent variants of *K. pneumoniae*, given the challenges they may pose in treatment or eradication efforts.^{14,97,113–117}

9 | CONCLUSIONS

This is the first extensive review of literature concerning venereal transmission for both *P. aeruginosa* and *K. pneumoniae*. Although both *P. aeruginosa* and *K. pneumoniae* are frequently referred to as venereal pathogens, data supporting this assertion are limited and often predated the advent of current methodologies including molecular typing technologies.^{13,14,18,56,63,73,93,107} The inconclusive evidence for *P. aeruginosa* and *K. pneumoniae* as important venereal pathogens strongly supports further investigation using molecular typing technologies to either confirm or exclude venereal spread of specific bacterial strains and genotypes amongst horses and facilities. The possibility of transmission of these bacteria by fomites or a common environmental source warrants additional investigation.^{12,78,98}

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Melanie Scholtz: Conceptualization; methodology; investigation; data curation; writing – original draft; writing – review and editing; visualization; project administration. **Alan John Guthrie:** Conceptualization; methodology; resources; writing – review and editing; supervision; funding acquisition. **Richard Newton:** Methodology; writing – review and editing; validation; supervision. **Martin Lance Schulman:** Conceptualization; methodology; writing – review and editing; visualization; supervision; project administration; funding acquisition.

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No new data were created or analysed in this study.

ETHICAL ANIMAL RESEARCH

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PEER REVIEW

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ORCID

Melanie Scholtz  <https://orcid.org/0000-0002-3198-2022>

Alan John Guthrie  <https://orcid.org/0000-0001-7729-9918>

Martin Lance Schulman  <https://orcid.org/0000-0003-0285-8679>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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