Emergence and Prevalence Decline of a Phenotypically Multidrug-Resistant *Staphylococcus pseudintermedius* in Israel

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ABSTRACT

The prevalence of multi-drug resistant *S. pseudintermedius* in Israel (IMDR), as assessed by a standardized disc-diffusion method, increased between 2004 and 2012 and decreased subsequently. Isolates were considered as IMDR if they were resistant to penicillin, oxacillin, ampicillin, amoxicillin–clavulanate, cephalothin, clindamycin, enrofloxacin, sulfamethoxazole-trimethoprim, gentamicin, erythromycin and tetracycline, or susceptible to one–tetracycline or sulfamethoxazole-trimethoprim. Isolates resistant to oxacillin were considered resistant to all beta-lactam antibiotics. The susceptibility to chloramphenicol and florfenicol was tested for 61 IMDR isolates of *S. pseudintermedius* to evaluate if phenicols may be considered as treatment options. Among them, 43 isolates were resistant to chloramphenicol. Inhibition zones for florfenicol (for which no interpretation standards for canine or feline isolates exist) were between 22 and 28 mm and between 18 and 21 mm for 59 and 2 isolates, respectively. IMDR still represents a challenge for veterinary medicine in Israel requiring further improvement of the standard hygienic procedures in veterinary settings as well as the search for alternative antimicrobial treatments. Puppies imported from Thailand may be a potential source of IMDR strains.

Keywords: Multidrug Resistant; *Staphylococcus pseudintermedius*; Israel; Animal Import; Florfenicol.

INTRODUCTION

Staphylococcus pseudintermedius is one of the most important opportunistic pathogens of dogs and cats, colonizing various parts of the skin such as the perineal area, nose, mouth and groin. It occasionally causes infections of these and other organs, usually as a result of predisposing factors. The microorganism's previous classification as *S. intermedius* was revised by molecular methods, resulting in several plasma coagulase positive species, isolated from different animals. *S. intermedius sensu stricto* was found exclusively in feral pigeons, *S. delphini* in marine mammals and other various animals. The type strain of *S. pseudintermedius* was isolated from the lung tissue of a cat and, unless proven otherwise, all *S. intermedius*-like isolates from dogs or cats are to be designed as *S. pseudintermedius* (1). *S. pseudintermedius* causes human infections very rarely but people in close contact with either colonized or infected animals may also carry genetically identical microorganism (2).

During the last decade, strains of methicillin-resistant *S. pseudintermedius* (MRSP) have been reported with increased frequency (3). In addition, these strains have been shown to be resistant to a number of other antibacterial drugs (4) and thus defined as multidrug resistant (MDR). Following genetic characterization, MRSP have been divided in several sequence types (ST) (5). The resistant strains belong primarily to 5 clonal complexes. While some of these were

originally limited to specific geographic areas, their worldwide distribution has increased (6). Animal transport might have contributed to this phenomenon.

The susceptibility of *S. pseudintermedius* to antibacterial drugs commonly used to treat dogs and cats in Israel has not changed significantly between 1990 and 2006 (Elad, unpublished data). Interestingly, the appearance of the IMDR strains coincides with the time frame of the reports of MDR strains emergence in other parts of the world.

In Israel, several isolates of *S. pseudintermedius* resistant to 10 or 11 of the 11 antibacterial drugs routinely tested *in vitro* were classified as ST45, a clonal lineage that has a high prevalence in Thailand (5). Each year, a variable number of puppies are regularly imported from Thailand to Israel, which may be a potential additional source of MDR *S. pseudintermedius*.

In this study we present a retrospective overview of the percentage of MDR of *S. pseudintermedius* isolated at the Laboratory of Clinical Bacteriology and Mycology at Kimron Veterinary Institute and phenotypically characterized by being resistant *in vitro* to 10 or 11 out of 11 antibacterial drugs routinely used in susceptibility testing of these microorganisms, and determine if imported puppies may carry MDR *S. pseudintermedius*.

MATERIALS AND METHODS

All the strains included in this survey were isolated from clinical samples from canines and felines (about 90% and 10% respectively) submitted to the Laboratory of Clinical Bacteriology an d Mycology at Kimron Veterinary Institute between 2003 and 2017 as well as from nasal and perineal swabs taken from 7 and 8 puppies in 2012 and 2013, respectively, collected at the airport quarantine station upon disembarking.

They were identified as belonging to the *Staphylococcus intermedius* complex based on standard methods (7) and defined as *S. pseudintermedius* if isolated from dogs or cats (1). Urinary tract isolates were excluded since they were tested with a different panel of antimicrobial drugs. *In vitro* susceptibility was assessed a disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) standards (8). When veterinary interpretation criteria were not available (for some of the additional drugs tested, not routinely used to treat animals), human standards for *S. aureus* were implemented. Results were measured with a digital imaging system (Biomic V3, Giles Scientific, USA) and recorded as inhibition zone diameters (allowing interpretation by the most recent standards). Antibacterial drugs routinely tested included penicillin, oxacillin, ampicillin, amoxicillin–clavulanate, cephalothin, clindamycin, enrofloxacin, sulfamethoxazole-trimethoprim, gentamicin, erythromycin and tetracycline. Strains exhibiting *in vitro* resistance to 10 or 11 of the 11 routinely tested drugs were classified as Israeli MDR (IMDR) *S. pseudintermedius* strains. In addition, 61 isolates, including the isolates from puppies, were also tested with chloramphenicol and florfenicol discs. Florfenicol is a drug used for bovine, pig and fish raising industries (9) where no accepted interpretive standards exist for disk-susceptibility results of *S. pseudintermedius* isolated from dogs or cats.

Moreover, ten random IMDR isolates were tested for susceptibility to amikacin, polymyxin B, fusidic acid, fosfomycin, linezolid, quinupristin/dalfopristin, rifampicin, tigecycline and vancomycin.

The mode is the most frequent value for a variable in a population. When a distribution curve has one mode peak (unimodal curve) it is likely that the variable assessed belongs one group. Each additional mode peak indicates that the population consists from an additional group with different values for the tested variable. Bimodal distribution curves of have two peaks and consequently indicate two groups, each with its own mode. We assessed the impact of the IMDR *S. pseudintermedius* isolates on the modality of the yearly MDR curves (the distribution of the number of antibacterial drugs to which *S. pseudintermedius* isolates were resistant) as previously described (10).

RESULTS

Clinical isolates exhibiting an IMDR resistance profile appeared for the first time in our laboratory in 2004. The rate of IMDR *S. pseudintermedius* isolates increased from 14.3% in 2008 to 32.7% in 2012, declining thereafter to 6.8% in 2017 (Figure 1). These strains were resistant to either all antibiotics tested routinely (penicillin, oxacillin, ampicillin, amoxicillin–clavulanate, cephalothin, clindamycin, enrofloxacin, sulfamethoxazole-trimethoprim, gentamicin, erythromycin and tetracycline) or only susceptible to either tetracycline or sulfamethoxazole-trimethoprim (Table 1). A few isolates were seemingly susceptible to cephalothin (2-6

Table 1: Yearly rates and susceptibility profiles of isolates susceptible to 0, or 1 out of 11 antibacterial drugs tested. MRSP isolates were considered resistant to all beta lactams.

Year (n)	None	Tet	SxT	Total
2003 (197)				
2004 (180)	1 (0.56)			1 (0.56)
2005 (193)				
2006 (241)	4 (1.7%)	2 (0.8%)		6 (2.5%)
2007 (155)		1 (0.9%)		1 (0.9%)
2008 (137)	6 (10.7%)	2 (3.6%)		8 (14.3%)
2009 (203)	27 (13.3%)	9 (4.6%)	3 (1.4%)	39 (19.3%)
2010 (212)	33 (15.8%)	2 (0.9%)	2 (0.9%)	37 (17.6%)
2011 (105)	8 (7.6%)	2 (1.9%)	10 (9.5%)	20 (19.0%)
2012 (150)	34 (22.7%)	5 (3.3%)	10 (6.7%)	49 (32.7%)
2013 (185)	34 (18.4%)	5 (2.7%)	7 (3.8%)	46 (24.9%)
2014 (126)	8 (6.3%)		5 (4.0%)	13 (10.3%)
2015 (137)	10 (7.3%)	2 (0.8%)	4 (2.9%)	16 (11.7%)
2016 (165)	5 (3.0%)	5 (3.0%)	10 (6.1%)	20 (12.1%)
2017 (103)	2 (1.9%)		5 (4.9%)	7 (6.8%)

n: total *S. pseudintermadius* isolates, Tet: tetracycline, SxT: sulfamethoxazole – trimethoprim

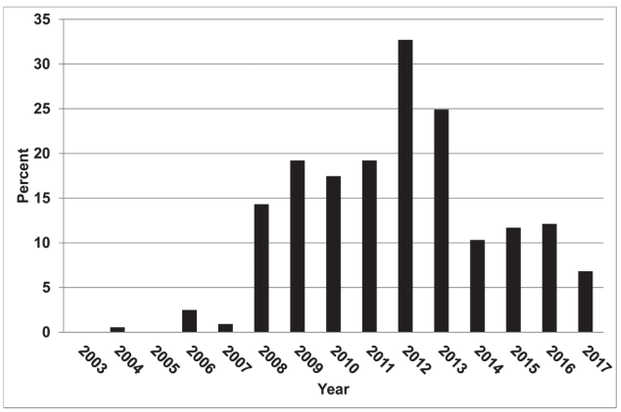

Figure 1: Yearly rates (percent of all *S. pseudintermedius* isolates) of IMDR isolates

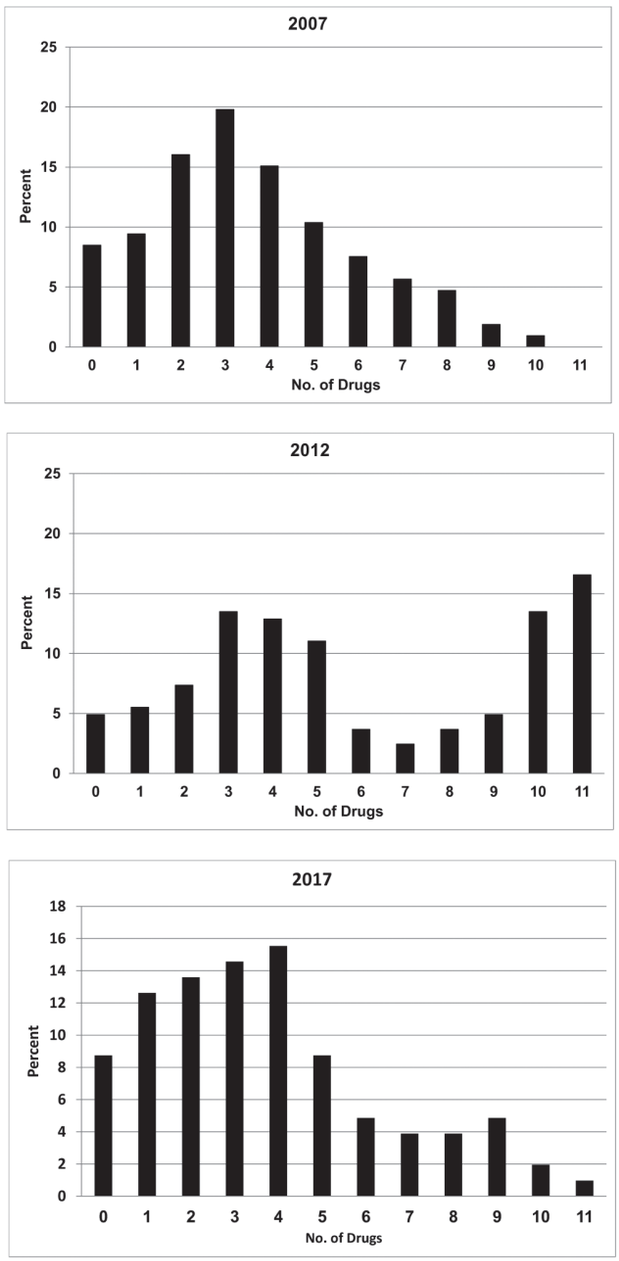

Figure 2: Distribution of *S. pseudintermedius* isolates by the number of drugs they were resistant to: emergence and regression of a bi-modal multi drug resistance curve indicating the presence of two discrete *S. pseudintermedius* populations with different multi drug-resistance characteristics.

isolates/year) and/or amoxicillin-clavulonic acid (0-1 isolate/year), but were considered resistant as per recommendation of the CLSI (8) for all oxacillin resistant strains. Neither canines nor felines were overrepresented in the population from which the isolates originated.

Plotting the rate of *S. pseudintermedius* isolates by the number of antibacterial drugs they were resistant to, a unimodal distribution in 2007, developing into a bimodal distribution in the following years. Bimodality declined and all but disappeared in 2017 (Figure 2).

No resistance to the human antibiotics amikacin, vancomycin, polymyxin B, fusidic acid, fosfomycin, linezolid, quinupristin/dalfopristin, rifampicin, and tigecycline was observed among a subset of ten IMDR isolates tested.

Of the imported puppies, 6 of 7 were found to carry MDR in 2012 and 3 of 8 in 2013. They exhibited a similar MDR resistance pattern as the IMDR.

Susceptibility testing of chloramphenicol and florfenicol of 61 MDR *S. pseudintermedius*, including 12 from the imported puppies, revealed that 40 (81.6%) Israeli isolates were resistant to chloramphenicol, 8 (16.3%) were susceptible and 1 (2.1%) showed an intermediate susceptibility. Of the 12 *S. pseudintermedius* strains isolated from imported pups, 3 (25%) were susceptible and 9 (75%) were resistant to chloramphenicol. Inhibition zone diameters for florfenicol were between 22 and 28 mm, with the exception of 2 isolates from imported puppies and two local isolates, with inhibition zones between 18 mm and 21 mm.

DISCUSSION

The definitions for Multi-Drug Resistant (MDR), Extended-Drug Resistant (XDR) and Pan-Drug Resistant (PDR) for several microorganisms, including *S. aureus*, have been published in the past (11). These criteria were based on resistance to drugs used in human medicine and no matching criteria exist for veterinary isolates. Since IMDR isolates were not resistant to all the drugs required to be defined as XDR or PDR, they were classified as MDR.

In this manuscript we report the emergence of a multi-drug resistant *S. pseudintermedius* in Israel, starting 2004. The source of these strains is not yet established. The rapid increase of IMDR has been so far attributed, at least partially, to a specific clone of the ST45 lineage (5), which is also prevalent in Thailand. Screening of a few puppies at the airport revealed that some of them animals were carriers of *S. pseudintermedius* isolates exhibiting an IMDR profile. Further molecular characterization of the strains is now necessary to determine the genetic relatedness between strains from Israel and from Thailand. Nevertheless, it is important to note that imported dogs may contribute to the global spread of MDR *S. pseudintermedius* and screening of puppies for epidemic MDR bacteria should be undertaken before export of these aniamls in order to limit the global dissemination of these bacteria.

The insurgence of MDR strains of *S. pseudintermedius* has severely limited the therapeutic options in many countries (3), including Israel. The topical use of miconazole (active on fungi and Gram positive bacteria) has been found to be beneficial (in concomitance with polymyxin B, an anti-Gram negative drug) in the treatment of infections associated with MDR *S. pseudintermedius*, especially affecting the ear (12). This drug, however, is not available for systemic use and further options need to be explored in order to avoid the use of antibiotics reserved for human treatment of staphylococcal infections.

The resistance of IMDR isolates to chloramphenicol and their apparent susceptibility to florfenicol is consistent with the latter being inactivated by fewer enzymes than the former (13). Florfenicol is currently limited to the treatment bovine, porcine and fish pathogens, with only very limited information regarding *in vitro* (9) and *in vivo* (14) use of the drug in dogs in general and against *S. pseudintermedius* specifically. Among the microorganisms with standardized CLSI breakpoints, the only Gram positive one is *Streptococcus suis* (22 mm and higher is considered susceptible), a value equaled or exceeded by all but 2 of our isolates. Although this cannot be considered an unequivocal indication of susceptibility, our results indicate that there are practically no therapeutical options for the treatment of infections caused by IMDR strains and thus this option should be explored further, including the definition of interpretation standards for florfenicol susceptibility testing of *S. pseudintermedius*.

CONFLICT OF INTEREST STATEMENT

The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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