Antibiotic resistance and genetic profile of *Klebsiella pneumoniae* from clinically diseased piglets

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Aim

In 2015, the first cases of septicaemia and sudden death in 2 to 3 week-old suckling piglets, resulting from *Klebsiella pneumoniae* subspecies *pneumoniae* (KPP) infection, were found in the Netherlands. In human medicine, KPP is notorious for carbapenemase production/multi-resistance and concomitant treatment difficulties. This project aimed to evaluate antibiotic resistance (ABR) and molecular profiles of KPP from diseased piglets.

Material & Methods

In total 22 KPP isolates from diseased piglets submitted for post-mortem examination were cultured at GD Animal Health. Antibiotic susceptibility testing (broth microdilution) was performed. MIC values were interpreted as susceptible or resistant (when applicable, resistant includes both 'intermediate susceptible' and 'resistant') according to breakpoints published by CLSI or national breakpoint committees or local breakpoints.
Additionally, eight isolates were subjected to a modified MLST using the genes *phoE*, *infB*, and *tonB*. After sequence analysis and alignment, concatenated sequences were used for constructing a maximum parsimony tree and sequence types (ST) were assigned.

Results

Phenotypically (n=22; 2015-2018), very low levels/absence of resistance were observed for amoxicillin/clavulanic acid, apramycin, cefepime, colistin, cefotaxime, enrofloxacin, flumequine, gentamicin, neomycin, streptomycin, tetracycline, trimethoprim and trimethoprim/sulfamethoxazole. A high level of resistance was observed for florfenicol and tilmicosin (see Table 1); KPP is regarded as intrinsically resistant to ampicillin, tiamulin, and tylosin.
KPP MLST showed that seven KPP isolates (including 3 isolates from the same farm) were genetically identical, sharing a known ST (ST30). For the other isolate another ST (ST37) was found (see Figure 1).

Discussion and Conclusion

The KPP isolates showed phenotypically (hardly) no resistance to antibiotics relevant for treatment of human cases of KPP. In this limited dataset, ST30 was the most common ST. A similar ST was found in English and Australian KPP isolates from diseased piglets with comparable clinical signs. However, identification of the STs does not correspond with previous published STs because in our MLST only 3 of the genes were used. Additional typing methods e.g. whole genome sequencing might give more insight in the genetic diversity of these isolates.
Also more KPP isolates from diseased piglets from different geographical areas need to be analysed to gain more insight into the dissemination of clinically relevant KPP strains and molecular evolution and distribution of KPP subtypes.

Figure 1. Results of the modified KPP MLST
Seven KPP isolates belonged to ST30; one isolate belonged to ST37. Circles represent isolates, larger circles represent more than one isolate with the same ST. Each colour represents another ST.

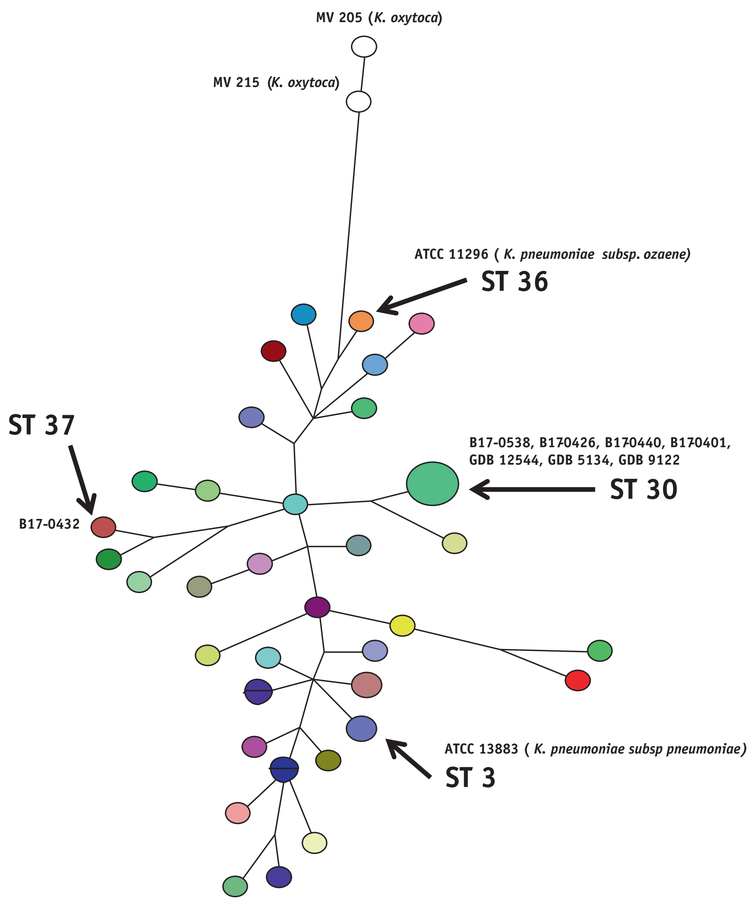

Table 1. MIC distribution (%) for *Klebsiella pneumoniae* subspecies *pneumoniae* isolates (n=22) originating from pigs submitted for post-mortem examination to the laboratory of GD AH, 2015 - 2018

Antibiotic	*Klebsiella pneumoniae* subspecies *pneumoniae (n=22)*											
	MIC-values (µg/mL)											
	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Amoxicillin/Clavulanic acid[a]	0.0	0.0	42.9	47.6	0.0	9.5	0.0	0.0	0.0			
Ampicillin	0.0	0.0	0.0	0.0	0.0	0.0	4.5	77.3	18.2			
Apramycin						100.0	0.0	0.0				
Cefepime			95.5	4.5	0.0	0.0	0.0	0.0	0.0			
Colistin		86.4	9.1	0.0	4.5	0.0	0.0	0.0				
Cefotaxime			100.0	0.0	0.0	0.0						
Enrofloxacin	100.0	0.0	0.0	0.0	0.0							
Florfenicol				0.0	54.5	40.9	4.5					
Flumequine				100.0	0.0	0.0	0.0	0.0				
Gentamicin				95.5	0.0	4.5	0.0					
Neomycin					95.5	4.5	0.0	0.0				
Sulfamethoxazole									27.3	31.8	4.5	36.4
Spectinomycin						0.0	0.0	81.8	4.5	4.5	9.1	
Streptomycin				81.8	4.5	0.0	4.5	4.5	0.0	4.5		
Tetracycline	0.0	4.5	50.0	31.8	0.0	0.0	0.0	13.6				
Tiamulin						0.0	0.0	0.0	100.0			
Tilmicosin				0.0	0.0	0.0	0.0	0.0	100.0			
Trimethoprim		86.4	0.0	0.0	0.0	0.0	0.0	13.6				
Trimethoprim/Sulfamethoxazole[b]	81.8	4.5	0.0	0.0	13.6	0.0						
Tylosin	0.0	0.0	0.0	0.0	0.0	100.0						

Dilution series applied for each individual antibiotic are marked green and red; green refers to the 'susceptible' and red to the 'resistant' range (where applicable, 'resistant' includes both 'intermediate susceptible' and 'resistant'). To the right of the dilution ranges shown in green and red, percentages of isolates with a MIC value higher than the highest concentration of the dilution range are mentioned in red. The percentage of isolates mentioned at the lowest concentration of a dilution range, refers to isolates with a MIC value equal to or lower than the lowest concentration evaluated in the specific dilution range.
[a] Only the concentration of amoxicillin, tested in a 2:1 ratio (amoxicillin : clavulanic acid) is mentioned;
[b] Only the concentration of trimethoprim, tested in a 1 :19 ratio (trimethoprim : sulfamethoxazole) is mentioned.

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