Identification of novel OXA-134-like β -lactamases in *Acinetobacter lwoffii* and *Acinetobacter schindleri* isolated from chicken litter

Patrik Mlynarcik^a, Jan Bardon^{a,c}, Miroslava Htoutou Sedlakova^a, Petra Prochazkova^a, Milan Kolar^{a,b}

Background. Various food-producing animals have been recognized in recent years as a potential reservoir for the spread of antibiotic resistant bacteria that may pose a risk to human health and therefore their dissemination in the food production chain needs to be assessed.

Methods. In this study, 450 boot swabs from chicken farms were analyzed for the presence of antimicrobial resistance with a focus on β -lactams resistance in *Acinetobacter* species.

Results. Two β -lactamase-encoding genes were first time identified in *Acinetobacter lwoffii* and *Acinetobacter schindleri* isolates. The deduced amino acid sequence of OXA-496 shared 93.8% identity with OXA-363. The second OXA-134-like enzyme, OXA-537, had the highest sequence identity (97.8%) with OXA-235 and OXA-237.

Conclusions. The results of this study illustrate the occurrence of new OXA-134-like β -lactamases, called OXA-496 and OXA-537, carrying strains of *Acinetobacter lwoffii* and *Acinetobacter schindleri* in chicken farm litter, and highlight the possible role of *Acinetobacter* as a reservoir of resistance genes.

Key words: antibiotics, chicken, β -lactamase, identification, resistance

Received: March 21, 2018; Accepted: June 22, 2018; Available online: July 18, 2018
<https://doi.org/10.5507/bp.2018.037>

^aDepartment of Microbiology, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic

^bInstitute of Molecular and Translation Medicine, Faculty of Medicine and Dentistry, Palacky University Olomouc, Czech Republic

^cState Veterinary Institute Olomouc, Czech Republic

Corresponding author: Patrik Mlynarcik, e-mail: patrik.mlynarcik@upol.cz

INTRODUCTION

Over the last decade, the prevalence of *Acinetobacter* spp. that are resistant to a number of commonly used antibiotics has increased. Resistance to β -lactams in *Acinetobacter* spp. can arise from (a) impaired permeability of the outer membrane via loss of porins; (b) up-regulation of efflux pumps; and/or (c) enzymatic inactivation of antibiotics. Important sources of antimicrobial resistance in *Acinetobacter* spp. arise from AmpC cephalosporinase¹, carbapenemases (within class A, GES- and KPC-types; within class B - members of the SIM, VIM, IMP, NDM; within class D, serine oxacillinases – OXAs) and other β -lactamases (e.g., CTX-M, VEB, PER, TEM and SHV types) (ref.²⁻¹⁰). So far, little data is available about the presence of intrinsic and/or acquired β -lactamases producing *Acinetobacter* spp. in food-producing animals; intrinsic bla_{OXA-51} -like genes (bla_{OXA-51} , $bla_{OXA-148}$, $bla_{OXA-149}$ and $bla_{OXA-150}$) from *Acinetobacter baumannii* in cattle and pig in Scotland, NDM-1-producing *A. baumannii* in pig in China and OXA-23-producing *Acinetobacter* species from horses and cattle have been detected^{4,6,11}, although OXA-23-like genes are intrinsic to *Acinetobacter radioresistens*¹². Further, the occurrence of bla_{NDM-1} -producing *Acinetobacter lwoffii* isolated from chickens in China has been reported². More recently, acquired extended-spectrum β -lactamases (ESBL), CTX-M-15 and TEM-116, have been reported in environmental *Acinetobacter* spp.¹³. This highlights the propen-

sity of *Acinetobacter* spp. to acquire different antibiotic resistance determinants.

Nosocomial infections caused by members of the genus *Acinetobacter* have been most often attributed to *A. baumannii*. However rarer, infections mainly associated with catheter-related bloodstream infections are caused by *A. lwoffii* and *Acinetobacter schindleri*¹⁴⁻¹⁶. Moreover, there is a growing concern over the transmission of ESBL and/or carbapenemase-producing *Acinetobacter* spp. in food producing animals (cattle, pig, horse and chicken) in many countries in Europe and Asia^{2,4,6}. Therefore, the role of healthy farm animals as a potential reservoir for the spread of antibiotic resistant bacteria and their impact on the food processing chain has to be assessed.

The aim of the current study was to investigate for the occurrence of β -lactams resistance in *Acinetobacter* species from chicken farm litter.

MATERIALS AND METHODS

Bacterial isolation

From November 2014 to March 2015, 450 boot swabs of farm staff were analyzed from chicken farms in Moravia (Czech Republic). Boot swabs were suspended in peptone water (Trios) and were grown aerobically at 37 °C for 24 h. A loopful of peptone water was streaked on Brilliance™ CRE agar plates (Oxoid). The agar plates were incubated for 24 h aerobically at 37 °C. The isolates

that grew were identified by matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) (Bruker Daltonik GmbH, Bremen, Germany) and were further tested for antibiotic susceptibility.

Twenty samples were taken from the 1) bedding material, 2) feed, 3) scrapings from the floor and 4) water before the poultry were placed and microbiologically examined in order to exclude microbial contamination in the chicken farm. Culture tests focused specifically on detection of *Acinetobacter* spp. Samples from the bedding material (1) and feed (2) were suspended in peptone water (Trios) and were grown aerobically at 37 °C for 24 h. A loopful of peptone water was streaked on Endo agar (Trios) and Brilliance™ CRE agar plates (Oxoid). The agar plates were incubated for 24 h aerobically at 37 °C. Disinfection control of hall floor prior to the housing of poultry was carried out by scraping the surface (100 cm²) using sterile swabs moistened with peptone water (3). The swab after delivery to the laboratory was incubated for 24 h in peptone water at 37 °C followed by inoculation of blood agar (Trios) and Endo agar (Trios), which were again incubated aerobically at 37 °C overnight. Water samples (4) for the detection of *Acinetobacter* spp. were examined by the modified method according to CSN EN/ISO 16266 (ref.¹⁷). This consisted of adding Endo agar (Trios) to the above mentioned solid culture media (Trios). Identification of isolates was performed in all cases after subculturing on blood agar by MALDI.

Antimicrobial susceptibility testing

Antibacterial drug susceptibility was performed using the broth microdilution and E-test (bioMérieux, France),

and interpreted according to CLSI document M100S (ref.¹⁸). The following antibiotics were tested: ampicillin, ampicillin/sulbactam, aztreonam, benzylpenicillin, cefalotin, cefazolin, cefepime, cefoperazone, cefoperazone/sulbactam, ceftazidime, cefuroxime, ciprofloxacin, cotrimoxazole, meropenem, oxacillin, piperacillin, piperacillin/tazobactam and tetracycline. For the E-test, a strip containing imipenem was used. All isolates were also tested for extended spectrum β -lactamase (ESBL) and AmpC β -lactamase using the ESBL + AmpC Screen kit (BioVendor).

Design of primers for PCR amplification

The sequences of genes encoding β -lactamases and carbapenemases (*bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM}, *bla*_{OXA}) so far described (<http://bldb.eu>; last accessed October 2017) (ref.¹⁹) were downloaded from the GenBank databases. Alignment was performed using Geneious Pro 8.1.9 (Biomatters, New Zealand) to identify homologous regions. Primers were then designed using Geneious software. In addition, a pair of flanking primers described by Kamolovit et al.²⁰ was used to amplify and sequence the entire *bla*_{OXA} gene. Detailed primer specifications are given in Table 2. The isolates were tested by PCR for the presence of β -lactamase and carbapenemase genes: *bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP}, *bla*_{NDM} and *bla*_{OXA}²¹⁻²³. Expression levels of the *bla*_{OXA} genes were not investigated.

DNA extraction and amplification conditions

Genomic DNA isolation was similar as described by Mlynarcik et al.²³. The PCR reaction mixture contained: 1 μ L DNA (100 ng) in 24 μ L complete reaction buffer

Table 1. Antimicrobial susceptibilities of the two *Acinetobacter* isolates.

Antibiotic(s)	MIC (mg/L) for isolate ^a :	
	<i>A. lwoffii</i> isolate S459	<i>A. schindleri</i> isolate S35
cotrimoxazole	>256 (R)	ND
tetracycline	16 (R)	8 (I)
ciprofloxacin	2 (I)	ND
ampicillin	<0.5 (S)	0.5 (S)
ampicillin/sulbactam	<0.5 (S)	0.5 (S)
cefepime	<0.2 (S)	0.2 (S)
meropenem	0.2 (S)	0.2 (S)
ceftazidime	1 (S)	1 (S)
piperacillin	2 (S)	2 (S)
cefalotin	8	ND
cefoperazone	8	8
cefazolin	16	32
aztreonam	4	8
cefoxitin	4	16
oxacillin	4	4
cefuroxime	1	2
benzylpenicillin	1	ND
cefoperazone/sulbactam	0.5 (S)	0.5 (S)
piperacillin/tazobactam	0.5 (S)	1 (S)
imipenem	0.125 (S)	0.125 (S)

^aSusceptibility categories are given in parentheses: I, intermediate; S, susceptible; R, resistant; ND, not defined.

Table 2. Sequences of primers used for PCR for detection of genes encoding β -lactamases and carbapenemases, and *rpoB* gene analysis.

Primer name	Sequence (5' to 3' direction) ^{a, b}	Target	Length (bases)	Amplicon size	T _m (°C)	Ref.
VIM-F	CGCGGAGATTGARAAGCAAA	41 VIM subtypes	20	247 bp	56	23
VIM-R	CGCAGCACCRGGATAGAARA		20			
NDM-F	GGGGATTGCGACTTATGC	15 NDM subtypes	18	258 bp	55	This study
NDM-R	AGATTGCCGAGCGACTTG		18			
IMP-1F	GAGTGGCTTAATTCTCRATC	40 IMP subtypes	20	183 bp	52	21, 22
IMP-1R	CCAAACYACTASGTTATCT		19			
IMP-2F	TGACGGGGTTAGTTATTGGCT	IMP-27	21	248 bp	56	This study
IMP-2R	CGGTTTCGCTATGACCTGAA		20			
OXA-1F	TGTACCTGCTTCGACCTTCA	113 OXA subtypes	20	436 bp	56	23
OXA-1R	TCCCCAACCACCTTTTTGCGT		20			
OXA-2F	ACGGAAAGCCAAGAGCCAT	21 OXA subtypes	19	358 bp	56	23
OXA-2R	CCCACACCAGAAAAACCAGT		20			
OXA-3F	ACTTGCTATGTGGTTGCTTCTC	22 OXA subtypes	22	310 bp	56	This study
OXA-3R	ACCTTTTCTCGCCCTTCCAT		20			
OXA-4F	GCAGAAAGAAGTAAAGCGGG	9 OXA subtypes	20	271 bp	56	This study
OXA-4R	CCAACCAGTCAACCAACCT		19			
OXA-5F	CCTCTCAAATCACTTCAACTCAATCT	5 OXA subtypes	26	253 bp	58	This study
OXA-5R	GCTGCAAACTGCGCTTCTT		20			
OXA-6F	ACGAGCACATACAGAATATGTCCC	5 OXA subtypes	24	510 bp	60	This study
OXA-6R	CCATTAGATTTTCTACCCAACCTGT		26			
Sequencing primers						
OXA-7F	GGCGAAGGTCAATCTCAAAA	flanking <i>bla</i> _{OXA-134-like} in <i>A. lwoffii</i>	20	1150 bp	56	20
OXA-7R	CGAGCAGACAGAGCAGAAGA		20			
Ac1005F	GTGATAARATGGCBGGTCGT	<i>rpoB</i> zone 2	20	543 bp	56	24
Ac1598R	CGBGCRTGCATYTTGTCRT		19			

^aFor degenerate primers: R = A or G; S = G or C; Y = C or T. ^bConcentration of each of the primers was 25 pmol.

with MgCl₂ (containing 100 mmol/L Tris-HCl [pH 8.8], 500 mmol/L KCl, 1% Triton X-100, 15 mmol/L MgCl₂) (Top-Bio, Czech Republic; 2.5 μ L), dNTP (10 mmol/L, 0.2 μ L), 25 pmol of each primer (0.2 μ L) and Taq DNA polymerase (Top-Bio, Czech Republic; 0.2 μ L). The PCR conditions were as follows: initial denaturation at 95 °C for 2 min, 30 cycles of 95 °C for 30 s, at different annealing temperatures for 30 s (according to Table 2) and 68 °C for 45-60 s, followed by a single, final, elongation step at 68 °C for 5 min. The primer sequences, annealing temperatures, and calculated lengths of the corresponding amplicons are listed in Table 2.

Identification of *Acinetobacter* species

Acinetobacter isolates were identified by MALDI and partial *rpoB* gene sequencing as described elsewhere²⁴. Sequences of *rpoB* gene (seqtypes) were included for analysis. Primers used for PCR amplification and sequencing are listed in Table 1. A neighbor-joining tree was constructed using the Geneious Tree Builder (Geneious Pro 8.1.9, Biomatters, New Zealand) using Tamura-Nei

Algorithm with 1000 bootstrap samplings. The *rpoB* gene sequence of *Pseudomonas aeruginosa* PAO1 (AE004091) was used as an outgroup. In comparative studies, sequences of *A. schindleri* strain LUH 5677 (KU961630), *A. schindleri* strain LUH 7428 (KU961631), *A. lwoffii* strain LMG 1300 (EF611398) and *A. lwoffii* strain LUH 1710 (KU961624) were also included.

GenBank accession numbers

The nucleotide sequences of *bla*_{OXA-496} from *A. lwoffii* isolate S459, *bla*_{OXA-537} from *A. schindleri* isolate S35, zone 2 of the *rpoB* gene from *A. lwoffii* isolate S459 and *A. schindleri* isolate S35 were deposited in the GenBank nucleotide database under accession numbers KT200608, KX360747, MF631202 (seqtype 2) and MF631203 (seqtype 1), respectively.

Epidemiological setting

Poultry litter in the halls was composed of thick wood shavings that normally do not come into contact with the soil, surface water or wild living animals. So if new litter

Fig. 1. Comparison of the amino acid sequences of the novel OXA-134-like variants (OXA-496 and -537), with the known OXA-134-like enzymes (OXA-363, -235 and -237), using the Geneious multiple sequence alignment program. Differences are highlighted with black letters.

material is brought into the hall, it is subjected to bacteriological investigation for any pathogenic bacteria. In order to prevent the entry of any potential disease and to reduce the stress of poultry, the movement of people in the hall is minimal. Feeding and water supply (municipal water) are controlled by automatic machines and they are regularly monitored for bacteriological contamination. The halls are protected against flying insects, wild birds and rodents by a biosecurity program. Workers and visitors are required to walk through disinfectant mats and must wear a clean lab coat and shoe covers. Biosecurity measures are strictly followed by farmers in order to reduce the risk of infections, especially avian influenza, Newcastle disease, and salmonellosis. The halls are mechanically cleaned and then disinfected after poultry leave the farm. The breeding hall is restocked only after satisfactory disinfection as determined from microbiological testing of swab samples from the floor.

RESULTS

A total of 55 Gram-negative bacilli were obtained from selective agar plates, from which 2 (3.6%), 3 (5.5%), 4 (7.3%), 6 (10.9%) and 9 (16.4%) isolates were identified as *A. radioresistens*, *Acinetobacter pittii*, *Acinetobacter calcoaceticus*, *A. lwoffii* and *A. schindleri*, respectively. Isolates of *Acinetobacter* species from the bedding material of chickens were subjected to susceptibility testing and screening for β -lactamase and carbapenemase production. However, the isolates exhibited high susceptibility to specific β -lactam antibiotics and the presence of studied resistance genes (*bla*_{KPC}, *bla*_{VIM}, *bla*_{IMP} and *bla*_{NDM}) has not been confirmed (data not shown). Further, the ESBL and AmpC β -lactamases were not detected in either strain. Interestingly, two *Acinetobacter* isolates were positive by

Fig. 2. Phylogenetic relationships between the two isolates (seqtype 1 – *Acinetobacter lwoffii* isolate S459 and seqtype 2 – *Acinetobacter schindleri* isolate S35) and other members of the genus *Acinetobacter* inferred from partial *rpoB* DNA sequences. Numbers at nodes represent bootstrap values out of 1000 resamplings that were greater than 50%. The tree was outgroup rooted with *Pseudomonas aeruginosa* strain PAO1 sequence.

using OXA-5F/R primers for detection of *bla*_{OXA-235 to -237/-278/-360} genes (See Table 2 for primer sets). Sequence analysis of the amplicon obtained from *A. lwoffii* isolate S459, generated with primers OXA-7F and OXA-7R (ref.²⁰), revealed the presence of a new member of the large OXA family and showed the highest amino acid identity to OXA-363 at 93.8% (Fig. 1) and was named OXA-496. *In silico* analysis revealed that the sequence located upstream (159 bp segment) had no insertion sequence element (<https://www-is.biotoul.fr/>) (data not shown). Sequence analysis of the amplicon obtained from *A. schindleri* isolate S35 showed the presence of a new variant of the OXA family, with the highest amino acid identity to OXA-134 family carbapenem hydrolyzing class D β -lactamase OXA-235 and OXA-237 at 97.8% (Fig. 1) and was named OXA-537. However, based on short sequence length of the up- and down-stream regions of the *bla*_{OXA} gene we were not able to predict an insertion sequence element.

Using the broth microdilution method, *A. lwoffii* isolate S459, was resistant to cotrimoxazole and tetracycline, had intermediate resistance to ciprofloxacin, and maintained susceptibility to ampicillin, ampicillin/sulbactam, cefepime, meropenem, ceftazidime and piperacillin (Table 1), based on the currently available breakpoints¹⁸. Another interesting isolate, *A. schindleri* isolate S35, was intermediately resistant to tetracycline, but maintained susceptibility to meropenem, cefepime, ampicillin, ampicillin/sulbactam, ceftazidime and piperacillin (Table 1). We determined the MICs of imipenem (0.125 μ g/mL) by using the E-test method which showed that the isolates were susceptible to carbapenem (Table 1).

Two *Acinetobacter* isolates from chicken bedding litter (S459 – seqtype 1 and S35 – seqtype 2) based on sequence analysis of zone 2 of the *rpoB* gene showed between 99.2%/99.1% and 99.8%/99.6% nucleotide identity to *Acinetobacter* type strains in GenBank, and were identified as: *A. lwoffii* ‘close to LMG 1300 and/or LUH 1710’ and *A. schindleri* ‘close to LUH 5677 and/or LUH 7428’, respectively. However, seqtype 1 showed different 3-bp or 4-bp sequence of partial *rpoB* gene with *A. lwoffii* strains LMG 1300 or LUH 1710, respectively (data not shown). The remaining seqtype 2 had only a 1-bp or 2-bp sequence difference between the *rpoB* genes of *A. schindleri* strains LUH 5677 or LUH 7428 (data not shown). The *Acinetobacter* isolates were further divided into two subgroups based on phylogenetic clustering (Fig. 2).

To exclude microbial contamination within the chicken farm, twenty samples were taken from the bedding material, feed, scrapings from the floor and water before the poultry were placed but no *Acinetobacters* were isolated in any of the samples.

DISCUSSION

All isolates of *Acinetobacter* species were subjected to susceptibility testing and screening of β -lactamase and carbapenemase production. Isolates showed high sensitivity to specific β -lactam antibiotics and did not confirm the

presence of the resistance genes examined. However, two isolates of *Acinetobacter* (*A. lwoffii* - S459 and *A. schindleri* - S35) from the chicken bedding litter were positive for two novel *bla*_{OXA-134-like} genes, *bla*_{OXA-496} and *bla*_{OXA-537}. These results can be explained by the absence of insertion sequence in upstream position of *bla*_{OXA-like} genes which provides the strong promoter sequence. It is therefore likely that *bla*_{OXA-134-like} genes were not expressed (or expressed at negligible level) in these hosts. In this study, the expression of the *bla*_{OXA-134-like} genes was not studied. Moreover, it has been described that the OXA-134-like enzymes are intrinsically present in *A. lwoffii* and *A. schindleri*^{25,26}. This has therefore proved to be a useful means of identification of these species. Although it could be a potential reservoir for antibiotic resistance. This suggests that our two new genes encoding OXA-134-like enzymes can be considered as intrinsic and unique to *A. lwoffii* and *A. schindleri*, but this has not been the subject of our study.

In this study, partial *rpoB* gene sequencing analysis revealed two isolates (S459 - seqtype 1 and S35 - seqtype 2) were *A. lwoffii* ‘close to LMG 1300’ and *A. schindleri* ‘close to LUH 5677’, respectively. Further, it has been reported that the *A. lwoffii* LMG 1300 can be isolated from soil and human clinical samples^{15,27}. However, *A. schindleri* LUH 5677 has been identified as animal-associated (isolated from trachea python) (ref.²⁸). Routine farm maintenance prevents poultry placement until halls are deemed microbial free. To support our findings twenty samples were taken from the bedding material, feed, scrapings from the floor and water before the poultry were placed and were sent to the microbiology laboratory for routine culture identification. However, in none of the samples were *Acinetobacters* isolated. These facts together lead us to believe that the strains of *Acinetobacter* could come from poultry but neither the environment nor human is excluded as a source of bacteria, as it is assumed impossible to have a chicken barn without microbes.

Acinetobacter are common, wild saprophytes found in soil, water, sewage and food of animal origin. *A. lwoffii* is one of the most prevalent *Acinetobacter* species involved in the economically important degradation of foods such as chicken, bacon, eggs, and fish, even when stored under cold conditions or after irradiation²⁹. However, there are many other pathogenic bacteria (e.g., *Pseudomonas* spp.) that cause degradation of food, particularly chicken meat. In the past, *Acinetobacter* species were considered saprophytes of little clinical significance. However, with the introduction of new potent antibiotics into clinical practice and agriculture, infections caused by resistant *Acinetobacter* species occur with increasing frequency^{10,13}. Thus, the identification of *Acinetobacter* species that carry different β -lactamases deserves continued attention.

The current epidemiology of OXA-producing bacteria in veterinary medicine is not yet fully understood, as data on the presence and distribution of these genes are lacking and therefore continuous monitoring is essential.

CONCLUSION

This is the first report describing novel OXA-134-like β -lactamases, OXA-496 and OXA-537, in *A. lwoffii* and *A. schindleri* isolated from chicken farm litter, and highlight the reservoir of resistance. Different emerging antimicrobial resistance properties in bacteria of food animal origin have been seen in the last years and further monitoring of the presence of resistance genes in these bacteria is warranted.

ABBREVIATIONS

ESBL, Extended-spectrum β -lactamases; MALDI MS, Matrix-assisted laser desorption/ionization mass spectrometry.

Acknowledgment: This publication was supported by the National Sustainability Program (LO1304), by the Palacky University Internal Financial Support, project no. IGA_LF_2018_019 and “Increasing internationalization of the Faculty of Medicine and Dentistry, Palacky University Olomouc” (SPP 210015017).

Author contributions: PM, MK, JB: study design; JB: collected the samples; PM, MHS, PP: laboratory work; All authors: contributed to the manuscript, read and approved the final manuscript.

Conflicts of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

REFERENCES

- Bou G, Martinez-Beltran J. Cloning, nucleotide sequencing, and analysis of the gene encoding an AmpC beta-lactamase in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2000;44(2):428-32.
- Wang Y, Wu C, Zhang Q, Qi J, Liu H, Wang Y, He T, Ma L, Lai J, Shen Z, Liu Y, Shen J. Identification of New Delhi metallo-beta-lactamase 1 in *Acinetobacter lwoffii* of food animal origin. *PLoS One* 2012;7(5):e37152.
- Pomba C, Endimiani A, Rossano A, Saial D, Couto N, Perreten V. First report of OXA-23-mediated carbapenem resistance in sequence type 2 multidrug-resistant *Acinetobacter baumannii* associated with urinary tract infection in a cat. *Antimicrob Agents Chemother* 2014;58(2):1267-8.
- Poirel L, Bercot B, Millemann Y, Bonnin RA, Pannaux G, Nordmann P. Carbapenemase-producing *Acinetobacter* spp. in Cattle, France. *Emerg Infect Dis* 2012;18(3):523-5.
- Smet A, Boyen F, Pasmans F, Butaye P, Martens A, Nemec A, Deschaght P, Vaneechoutte M, Haesebrouck F. OXA-23-producing *Acinetobacter* species from horses: a public health hazard? *J Antimicrob Chemother* 2012;67(12):3009-10.
- Zhang W-J, Lu Z, Schwarz S, Zhang R-M, Wang X-M, Si W, Yu S, Chen L, Liu S. Complete sequence of the bla(NDM-1)-carrying plasmid pNDM-AB from *Acinetobacter baumannii* of food animal origin. *J Antimicrob Chemother* 2013;68(7):1681-2.
- Pasteran F, Rapoport M, Petroni A, Faccione D, Corso A, Galas M, Vazquez M, Procopio A, Tokumoto M, Cagnoni V. Emergence of PER-2 and VEB-1a in *Acinetobacter baumannii* Strains in the Americas. *Antimicrob Agents Chemother* 2006;50(9):3222-4.
- Endimiani A, Luzzaro F, Migliavacca R, Mantengoli E, Hujer AM, Hujer KM, Pagani L, Bonomo RA, Rossolini GM, Toniolo A. Spread in an Italian hospital of a clonal *Acinetobacter baumannii* strain producing the TEM-92 extended-spectrum beta-lactamase. *Antimicrob Agents Chemother* 2007;51(6):2211-4.
- Naas T, Namdari F, Reglier-Poupet H, Poyart C, Nordmann P. Panresistant extended-spectrum beta-lactamase SHV-5-producing *Acinetobacter baumannii* from New York City. *J Antimicrob Chemother* 2007;60(5):1174-6.
- Nagano N, Nagano Y, Cordevant C, Shibata N, Arakawa Y. Nosocomial transmission of CTX-M-2 beta-lactamase-producing *Acinetobacter baumannii* in a neurosurgery ward. *J Clin Microbiol* 2004;42(9):3978-84.
- Hamouda A, Findlay J, Al Hassan L, Amyes SGB. Epidemiology of *Acinetobacter baumannii* of animal origin. *Int J Antimicrob Agents* 2011;38(4):314-8.
- Poirel L, Figueiredo S, Cattoir V, Carattoli A, Nordmann P. *Acinetobacter radioresistens* as a silent source of carbapenem resistance for *Acinetobacter* spp. *Antimicrob Agents Chemother* 2008;52(4):1252-6.
- Maravic A, Skocibusic M, Fredotovic Z, Samanic I, Cvjetan S, Knezovic M, Puizina J. Urban riverine environment is a source of multidrug-resistant and ESBL-producing clinically important *Acinetobacter* spp. *Environ Sci Pollut Res Int* 2016;23(4):3525-35.
- Seifert H, Strate A, Schulze A, Pulverer G. Vascular catheter-related bloodstream infection due to *Acinetobacter johnsonii* (formerly *Acinetobacter calcoaceticus* var. *lwoffii*): report of 13 cases. *Clin Infect Dis* 1993;17(4):632-6.
- Dortet L, Legrand P, Soussy C-J, Cattoir V. Bacterial identification, clinical significance, and antimicrobial susceptibilities of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *J Clin Microbiol* 2006;44(12):4471-8.
- Dijkshoorn L, Nemec A, Seifert H. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* 2007;5(12):939-51.
- CSN EN/ISO 16266. Water quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration. Czech Standards Institute; 2008.
- CLSI. Performance standards for antimicrobial susceptibility testing; twenty-sixth informational supplement. Wayne, PA: Clinical Laboratory and Standards Institute; 2016.
- Naas T, Oueslati S, Bonnin RA, Dabos ML, Zavala A, Dortet L, Retailleau P, Iorga BI. Beta-lactamase database (BLDB) - structure and function. *J Enzym Inhib Med Ch* 2017;32(1):917-9.
- Kamolovit W, Higgins PG, Paterson DL, Seifert H. Multiplex PCR to detect the genes encoding naturally occurring oxacillinases in *Acinetobacter* spp. *J Antimicrob Chemother* 2014;69(4):959-63.
- Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. *J Antimicrob Chemother* 2012;67(4):906-9.
- Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *J Antimicrob Chemother* 2007;59(2):321-2.
- Mlynarcik P, Roderova M, Kolar M. Primer Evaluation for PCR and its Application for Detection of Carbapenemases in *Enterobacteriaceae*. *Jundishapur J Microbiol* 2016;9(1).
- La Scola B, Gundi VA, Khamis A, Raoult D. Sequencing of the rpoB gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol* 2006;44(3):827-32.
- Turton JF, Hyde R, Martin K, Shah J. Genes encoding OXA-134-like enzymes are found in *Acinetobacter lwoffii* and *A. schindleri* and can be used for identification. *J Clin Microbiol* 2012;50(3):1019-22.
- Figueiredo S, Poirel L, Seifert H, Mugnier P, Benhamou D, Nordmann P. OXA-134, a naturally occurring carbapenem-hydrolyzing class D beta-lactamase from *Acinetobacter lwoffii*. *Antimicrob Agents Chemother* 2010;54(12):5372-5.
- Baumann P. Isolation of *Acinetobacter* from soil and water. *J Bacteriol* 1968;96(1):39-42.
- Van Assche A, Alvarez-Perez S, de Breij A, De Brabanter J, Willems KA, Dijkshoorn L, Lievens B. Phylogenetic signal in phenotypic traits related to carbon source assimilation and chemical sensitivity in *Acinetobacter* species. *Appl Microbiol Biotechnol* 2017;101(1):367-79.
- Bergogne-Bérézin E, Joly-Guillou ML, Towner KJ. *Acinetobacter*: microbiology, epidemiology, infections, management. Boca Raton: CRC Press, 1996.