

Original Research article

Relevance and epidemiology of *Achromobacter xylosoxidans* from non-respiratory tract clinical samples: a 10-year retrospective study in a tertiary-care hospital in Hungary

ABSTRACT

Aims: To assess the prevalence of *A. xylosoxidans* isolated from non-respiratory tract samples from adult inpatients and outpatients and the antibiotic resistance levels at a tertiary-care teaching hospital in Szeged, Hungary retrospectively, during a 10-year study period.

Study design: Retrospective microbiological study.

Place and Duration of Study: 1st of January 2008 - 31st of December 2017 at the University of Szeged, which is affiliated with the Albert Szent-Györgyi Clinical Center, a primary- and tertiary-care teaching hospital in the Southern Great Plain of Hungary.

Methodology: Data collection was performed electronically. Antimicrobial susceptibility testing (AST) was performed using disk diffusion method and when appropriate, E-tests on Mueller–Hinton agar (MHA) plates.

Results: During the 10-year study period, a total of 68 individual *A. xylosoxidans* isolates were identified (6.8 ± 3.6 /year, range: 0-11 isolates). The frequency of isolation in the first half of the study period (2008-2012) was $n=22$, while in 2013-2017, this number was $n=46$. The majority of isolates (51 out of 68) were from inpatient departments. 32 out of 68 patients were female (female-to-male ratio: 0.89). The susceptibilities of the respective *A. xylosoxidans* isolates ($n=68$) were the following: high levels of susceptibility for IMP and MER ($n=63$; 92.6%), and MOX ($n=55$; 80.9%), while higher rates of resistance were detected for SXT (susceptible: $n=36$; 52.9%), CIP (susceptible: $n=40$; 58.8%) and almost all isolates were resistant to CZD (susceptible: $n=3$; 4.4%) and FEP ($n=2$; 2.9%).

Conclusion: The existing literature on *Achromobacter* infections in the context of non-respiratory human infections is scarce, as the incidence of these pathogens in clinically-relevant syndromes is low. The developments in diagnostic technologies in routine clinical microbiology will probably lead to a shift in the isolation frequency of these bacteria in the future.

Keywords: *Achromobacter xylosoxidans*, non-fermenting, Gram-negative, epidemiology, immunocompromised, retrospective, clinical microbiology, medicine

1. INTRODUCTION

The genus *Achromobacter* includes lactose-non-fermenting Gram-negative bacteria that are aerobic, motile (with peritrichous flagella), oxidase and catalase-positive [1]. Taxonomically, these bacteria are the members of the *Alcaligenaceae* family of the Burkholderiales order; based on their genome sequences, these bacteria are most similar to *Bordetella* species [2]. In fact, until recently, the genus *Achromobacter* was specified into the *Alcaligenes* genus [3]. The members of the genus are ubiquitous, their isolation has been reported from soil samples, water reservoirs and from plants [4]. From the context of clinical samples, *A. xylosoxidans* is the most frequently isolated species; the isolation of this pathogen has been described from blood, stool, urine, cerebrospinal fluid, peritoneal fluid, sputum, ear discharge, abscesses, bone and joint samples and central venous catheters [5]. However, the most clinical data to date have been collected on *A. xylosoxidans* pneumonia in cystic fibrosis (CF) patients [6-7]. The prevalence of this pathogen in the sputum of CF-patients is estimated to be around 2-25%, and co-infection or co-isolation with *Pseudomonas aeruginosa* is very common [8]. In lung transplant patients with CF, it was observed that the pan-resistant (PDR) *A. xylosoxidans* was present before transplantation, and that this PDR *A. xylosoxidans* recurred in one-third of patients after transplantation [9]. However, colonization with *A. xylosoxidans* did not correlate with post-transplant survival and should not be considered as a reason for transplant rejection in the US, but the decrease in lung function after transplantation showed correlation with the presence of this bacterium [10].

Most of the reported cases of non-CF *A. xylosoxidans* infections are nosocomial infections in immunocompromised hosts: the source of the infection may be the indwelling catheters, endotracheal tubes or other invasive medical devices [11]. In addition, the gastrointestinal tract has been suspected as a source of invasive infection, where the increased permeability of the mucosal barrier may lead to disseminated infections, such as sepsis and meningitis [12]. The most numerous cases in adults have been reported in patients with malignancies, HIV-infection, neutropenia, bone marrow transplant, IgM-deficiency and high-dose corticosteroid therapy, while pre-term delivery is an independent risk factor in infants [4-13]. Therefore, *Achromobacter* spp. are recognized as emerging pathogens that can cause infections in patients with impaired immune system and are well-known nosocomial pathogens, especially in the intensive care units (ICUs) [5]. However, clinicians often are uninformed about the microbiology and clinical relevance of these bacteria and dismiss them as contaminants.

The epidemiology and antibiotic susceptibility-patterns of pathogens vary greatly by region; therefore, the assessment of local data is essential to evaluate trends over time and to reflect on the national situation compared to international data. With this in mind, the aim of this study was to assess the prevalence of *A. xylosoxidans* isolated from non-respiratory tract samples from adult inpatients and outpatients and the antibiotic resistance levels at a tertiary-care teaching hospital in Szeged, Hungary retrospectively, during a 10-year study period.

2. METHODOLOGY

2.1. LOCATION AND POPULATION OF THE STUDY, DATA COLLECTION

During our study, the laboratory information system of the Institute of Clinical Microbiology (University of Szeged) was searched for samples positive for *A. xylosoxidans*, corresponding to the time period between 2008.01.01.–2017.12.31 (10 years). The Institute is the primary microbiological diagnostic laboratory of the Albert Szent-Györgyi Clinical Center, providing medical care for a population of around 600,000 people, based on the most recent census data [15]. Data collection was performed electronically, based on the following criteria: samples with significant colony counts for *A. xylosoxidans* ($>10^5$ CFU/mL for urine samples, while $>10^3$ in case of other types of clinically-relevant samples; however, this was subject to interpretation by the senior clinical microbiologists, based on the information provided on the clinical request forms for the microbiological analysis and international guidelines) [15]. Respiratory samples were excluded from this analysis. Only the first isolate per patient was included in the study; however, isolates with different antibiotic-susceptibility patterns from the same patient were considered as different individual isolates. To evaluate the demographic characteristics of these infections, patient data was also collected, which was limited to sex, age at sample submission, and inpatient/outpatient status of patients over 18 years of age. The immune status of the patients or their underlying illnesses were not known during the study.

2.2. SAMPLE PROCESSING AND IDENTIFICATION

The processing of relevant samples arriving to the Institute of Clinical Microbiology was carried out according to guidelines in routine clinical bacteriology. Between 2008–2012, the BD Bactec (Beckton Dickinson, Franklin Lakes, NJ, USA) detection system was employed for the incubation of blood culture bottles, whilst from 2013 onwards, the BacT/ALERT 3D (bioMérieux, Marcy-l'Étoile, France) detection system was used. Blood culture bottles were incubated for 5 days (21 days, if endocarditis was suspected) in the abovementioned detection systems. The processing of urine samples was as follows: 10 μ L of each un-centrifuged urine sample was cultured on UriSelect chromogenic agar plates (Bio-Rad, Berkeley, CA, USA) with a calibrated loop, according to the manufacturer's instructions and incubated at 37 °C for 24–48 h, aerobically. The workup of faecal samples was performed on the appropriate non-selective and selective media, relevant to the isolation of diarrheal pathogens. If the relevant pathogens presented in significant colony count, the plates were passed on for further processing [16].

Between 2008–2012, presumptive phenotypic (biochemical reaction-based) methods and VITEK 2 ID (bioMérieux, Marcy-l'Étoile, France) were used for bacterial identification, while after 2013, this was complemented by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonik GmbH, Gr., Bremen, Germany). Bacterial cells were transferred to a stainless-steel target. An on-target extraction was performed by adding 1 μ l of 70% formic acid prior to the matrix. After drying at ambient temperature, the cells were

covered with 1 µl matrix (α -cyano-4-hydroxy cinnamic acid in 50% acetonitrile/2.5% trifluoro-acetic acid). Mass spectrometry was performed by the Microflex MALDI Biotyper (Bruker Daltonics, Bremen, Germany) in positive linear mode across the m/z range of 2 to 20 kDa; for each spectrum, 240 laser shots at 60 Hz in groups of 40 shots per sampling area were collected. The MALDI Biotyper RTC 3.1 software (Bruker Daltonics) and the MALDI Biotyper Library 3.1 were used for spectrum analysis [17].

2.3. ANTIMICROBIAL SUSCEPTIBILITY TESTING (AST)

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method and when appropriate, E-test (Liofilchem, Abruzzo, Italy) on Mueller–Hinton agar (MHA) plates. The interpretation of the results was based on EUCAST breakpoints for *Pseudomonas* spp. and *Acinetobacter* spp. (when relevant). The following antibiotics were tested: piperacillin/tazobactam (TZP), ceftazidime (CZD), cefepime (FEP), imipenem (IMP), meropenem (MER), ciprofloxacin (CIP), moxifloxacin (MOX) and sulfamethoxazole/trimethoprim (SXT). Colistin (COL) susceptibility was performed using the broth microdilution method in a cation-adjusted Mueller-Hinton broth (MERLIN Diagnostik). Colistin susceptibility testing was not routinely performed, only per request of the clinicians. During data analysis, intermediately-susceptible results were grouped with and reported as resistant. Classification of the isolates as a multidrug resistant (MDR) or extensively drug resistant (XDR) was based on the EUCAST Expert Rules [18]. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

2.4. STATISTICAL ANALYSIS

Descriptive statistical analysis (including means or medians with ranges and percentages to characterize data) was performed using Microsoft Excel 2013 (Redmond, WA, Microsoft Corp.).

2.5. ETHICAL CONSIDERATIONS

The study was deemed exempt from ethics review by the Institutional Review Board and informed consent was not required as data anonymity was maintained.

3. RESULTS AND DISCUSSION

3. 1. EPIDEMIOLOGY OF *A. XYLOSOXIDANS*

During the 10-year study period, a total of 68 individual *A. xylosoxidans* isolates were identified (6.8 ± 3.6 /year, range: 0-11 isolates; highest in 2016, lowest in 2009 and 2011) from non-respiratory tract samples. The frequency of isolation in the first half of the study period (2008-2017) was $n=22$, while in 2013-2017, this number was $n=46$. It must be noted that $n=12$ non-*xylosoxidans* *Achromobacter*

species have also been isolated, however, these were excluded from this data analysis.

3.2. DEMOGRAPHIC CHARACTERISTICS

The majority of isolates (51 out of 68) were from inpatient departments, namely the Intensive Care Department (n=23), Department of Traumatology (n=14), Department of Internal Medicine (n=6), Department of Immunology and Allergology (n=5) and Department of Neurology (n=3); the rest n=17 of the isolates came from various outpatient clinics. *A. xylosoxidans* was isolated from the following samples types: urine (midstream and catheterized): n=26, blood cultures: n=17, central venous catheters: n=8, faeces: n=7, biopsy samples: n=6, puncture samples: n=4, respectively. No dominance regarding the distribution of patients were observed towards either sexes: 32 out of 68 patients were female (female-to-male ratio: 0.89); the age distribution of patients was the following: 18-35 years: n=9, 36-59 years: n=15 and 60 years or older: n=44.

3.3. ANTIMICROBIAL SUSCEPTIBILITY

The susceptibilities of the respective *A. xylosoxidans* isolates (n=68) were the following: high levels of susceptibility for IMP and MER (n=63; 92.6%), and MOX (n=55; 80.9%), while higher rates of resistance were detected for SXT (susceptible: n=36; 52.9%), CIP (susceptible: n=40; 58.8%) and almost all isolates were resistant to CZD (susceptible: n=3; 4.4%) and FEP (n=2; 2.9%). COL susceptibility was performed in n=10 cases, all isolates were susceptible. Based on AST results, n=4 (5.9%) could be considered as MDR, while no XDR isolates were detected.

3.4. DISCUSSION

The present study reports on the epidemiological features of *A. xylosoxidans* infections at a tertiary-care hospital in Hungary over a period of a decade (2008-2017). Although the relevance of this pathogen is emerging and its isolation and identification is becoming more frequent, little is known regarding the virulence characteristics of *Achromobacter*, especially the ones concerning its ability to adhere, colonize and subsequently cause infections *in vivo* [19,20]. The following virulence determinants have been identified in the genus: flagella, lipopolysaccharide (LPS), other membrane-associated structures, phospholipase C, various proteases, cellulose, a type-3 secretion system (T3SS); these have all been noted to have roles in the inflammatory reaction caused by this bacteria in the airways, however, their roles in invasive infections are not yet understood [19-21]. In addition, the bacteria possess the ability to denitrify, thus, allowing for their persistence and survival in hypoxic or anaerobic environments [2-4]. The production of biofilm is another significant factor in the pathogenicity and survival of *A. xylosoxidans* in both respiratory infections and catheter-associated infections [22]. The reported mortality rate in invasive *Achromobacter* infections is around 2% for bacteremia, while this may reach 80% in case of neonatal sepsis [4-13].

Similarly to other non-fermenting Gram-negative bacteria, *Achromobacter* species have a plethora of intrinsic resistance mechanisms: penicillins, 1-2nd generation cephalosporins, chloramphenicol, macrolides and aminoglycosides [4-13,17,19,20]. Fluoroquinolones are usually considered as the part of a combination regimen (not as monotherapy) with carbapenems, therefore IMP, MER, TZP and SXT are the drugs of choice in these infections, preferably in combination. COL remains a viable alternative in case of extensive resistance, however, there are limited clinical experiences with this drugs against *Achromobacter* species [23]. In addition, due to the genetic plasticity of these microorganisms, they may also facilitate horizontal gene transfer between bacteria, promoting the spread of antimicrobial resistance. The over-expression of bacterial efflux pumps is another significant resistance mechanism in this pathogen, mainly affecting susceptibilities for the fluoroquinolones. Resistance against β -lactams has also been noted, both in the form of intrinsic ($bla_{OXA-144}$ -like, bla_{OXA-2} , $bla_{OXA-243}$), inducible (AmpC enzymes) and plasmid-mediated (bla_{IMP}) β -lactamases, and the effects of efflux pumps were also associated with β -lactam resistance [5]. In these cases, the therapeutic armamentarium for these infections narrows significantly [25].

4. CONCLUSION

The existing literature on *Achromobacter* infections in the context of non-respiratory human infections is scarce, as the incidence of these pathogens in clinically-relevant syndromes is low. It should be noted, that the difficulty in the adequate identification (especially in low-resource settings) may be partly blamed for the infrequent characterization of these bacteria as significant pathogens. Nevertheless, the developments in diagnostic technologies in routine clinical microbiology (e.g., MALDI-TOF MS) will probably lead to a shift in the isolation frequency of these bacteria in the future. Due to these technical developments in routine microbiology, the prevalence of bacterial species that were previously considered as rare will most probably increase, which is reflected in the increase in the interest towards these bacteria in the literature and our present report.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

The study was deemed exempt from ethics review by the Institutional Review Board, and informed consent was not required as data anonymity was maintained.

REFERENCES

1. Vandamme P, Moore ERB, Cnockaert M, De Brandt E, Svensson-Stadler L, Houf K, Spilker T, LiPuma JJ. *Achromobacter animicus* sp. nov., *Achromobacter mucicolens* sp. nov., *Achromobacter pulmonis* sp. nov. and *Achromobacter spiritinus* sp. nov., from human clinical samples. *Syst Appl Microbiol* 2013; 36:1–10.
2. Gomilla M, Prince-Manzano C, Svensson-Stadler L, Busquets A, Erhardt M, Martínez DL, Lalucat J, Moore ERB. Genotypic and Phenotypic Applications for the Differentiation and Species-Level Identification of *Achromobacter* for Clinical Diagnoses. *Plos One* 2014; 9: e114356.
- 3.
4. Ridderberg W, Bendstrup KEM, Olesen HV, Jensen-Fangel S, Nørskov- Lauritsen N. Marked increase in incidence of *Achromobacter xylosoxidans* infections caused by sporadic acquisition from the environment. *J Cyst Fibros* 2011; 10:466–469

4. Busse HJ., Stolz A. (2006) *Achromobacter*, *Alcaligenes* and Related Genera. In: Dworkin M., Falkow S., Rosenberg E., Schleifer KH., Stackebrandt E. (eds) *The Prokaryotes*. Springer, New York, NY.
5. Krzewinski JW, Nguyen CD, Foster JM, Burns JL. 2001. Use of random amplified polymorphic DNA PCR to examine epidemiology of *Stenotrophomonas maltophilia* and *Achromobacter* (*Alcaligenes*) *xylosoxidans* from patients with cystic fibrosis. *J Clin Microbiol* 2001; 39: 3597–3602.
6. de Baets F, Schelstraete P, Van Daele S, Haerynck F, Vaneechoutte M. *Achromobacter xylosoxidans* in cystic fibrosis: prevalence and clinical relevance. *J Cyst Fibros* 2007; 6:75–78.
7. Amoureux L, Bador J, Siebor E, Taillefumier N, Fanton A, Neuwirth C. Epidemiology and resistance of *Achromobacter xylosoxidans* from cystic fibrosis patients in Dijon, Burgundy: first French data. *J Cyst Fibros* 2013; 12:170–176.
8. Cools P, Ho E, Vranckx K, Schelstraete P, Wurth B, Franckx H, Ieven G, Van Simaey L, Van Daele S, Verhulst S, De Baets F, Vaneechoutte M. Epidemic *Achromobacter xylosoxidans* strain among Belgian cystic fibrosis patients and review of literature. *BMC Microbiol* 2016; 16:122.
9. Moissenet D, Baculard A, Valcin M, Marchand V, Tournier G, Garbarg-Chenon A, Vu-Thien H. 1997. Colonization by *Alcaligenes xylosoxidans* in children with cystic fibrosis: a retrospective clinical study conducted by means of molecular epidemiological investigation. *Clin Infect Dis* 1997; 24: 274–275.
10. Block JK, Vandemheen KL, Tullis E, Fergusson D, Doucette S, Haase D, Berthiaume Y, Brown N, Wilcox P, Bye P, Bell S, Noseworthy M, Pedder L, Freitag A, Paterson N, Aaron SD. Predictors of pulmonary exacerbations in patients with cystic fibrosis infected with multi-resistant bacteria. *Thorax* 2006; 61:969–974.
11. Ahmed MS, Nistal C, Jayan R, Kuduvalli M, Anijeet HK. *Achromobacter xylosoxidans*, an emerging pathogen in catheter-related infection in dialysis population causing prosthetic valve endocarditis: a case report and review of literature. *Clin Nephrol* 2009; 71: 350-354.
12. Schoch PE, Cunha BA. Nosocomial *Achromobacter xylosoxidans* infections. *Infect Control Hosp Epidemiol* 1988; 9: 84-87.
13. Weitkamp JH, Tang YW, Hass DW, Midha NK, Crowe JE. Recurrent *Achromobacter xylosoxidans* Bacteremia Associated with Persistent Lymph Node Infection in a Patient with Hyper-Immunoglobulin M Syndrome. *Clin Infect Dis* 2000; 31: 1183-1187.
14. Al-Jasser MA, Al-Anazi KA. Complicated septic shock caused by *Achromobacter xylosoxidans* bacteremia in a patient with acute lymphoblastic leukaemia. *Lybian J Med* 2007; 2: 218-219.
15. Gajdács M, Urbán E. Resistance Trends and Epidemiology of *Citrobacter-Enterobacter-Serratia* in Urinary Tract Infections of Inpatients and Outpatients (RECESUTI): A 10-Year Survey. *Medicina (Kaunas)* 2019; 55: e285.
16. Gajdács M. Epidemiology and antibiotic resistance trends of *Pantoea* species in a tertiary-care teaching hospital: A 12-year retrospective study. *Dev Health Sci* 2019; 2: 72-75.
17. Gajdács M, Burián K, Terhes G. Resistance Levels and Epidemiology of Non-Fermenting Gram-Negative Bacteria in Urinary Tract Infections of Inpatients and Outpatients (RENFUTI): A 10-Year Epidemiological Snapshot. *Antibiotics* 2019; 8: e143.
18. Leclercq, R.; Cantón, R.; Brown, D.F.J.; Giske, C.G.; Heisig, P.; MacGowan, A.P.; Mouton, J.W.; Nordmann, P.; Rodloff, A.C.; Rossolini, G.M.; et al. EUCAST expert rules in antimicrobial susceptibility testing. *Clin. Microbiol. Infect.* 2013, 19, 141–160.
19. Pereira RH, Leão RS, Carvalho-Assef AP, Albano RM, Rodrigues ER, Firmida MC, Folescu TW, Plotkowski MC, Bernardo VG, Marques EA. Patterns of virulence factor expression and antimicrobial resistance in *Achromobacter xylosoxidans* and *Achromobacter ruhlandii* isolates from patients with cystic fibrosis. *Epidemiol Infect* 2017; 145: 600-606.

20. Filipic B, Malesevic M, Vasiljevic Z, Lukic J, Novovic K, Kojic M, Jovic B. Uncovering Differences in Virulence Markers Associated with *Achromobacter* Species of CF and Non-CF Origin. *Front Cell Infect Microbiol* 2017; 7: 224.
21. Edwards BD, Greysen-Wong J, Somayaji R, Waddell B, Whelan FJ, Storey DG, Rabin, HR, Surette MG, Parkins MD. Prevalence and Outcomes of *Achromobacter* Species Infections in Adults with Cystic Fibrosis: a North American Cohort Study. *J Clin Microbiol* 2017; 55: 2074-2085.
22. Konstantinović N, Ćirković I, Đukić S, Marić V, Božić DD. Biofilm formation of *Achromobacter xylosoxidans* on contact lens. *Acta Microbiol Immunol Hung*. 2017; 64: 293-300.
23. Biswas S, Dubus JC, Reynaud-Gaubert M, Stremmer N, Rolain JM. Evaluation of colistin susceptibility in multidrug-resistant clinical isolates from cystic fibrosis, France. *Eur J Clin Microbiol Infect Dis* 2013; 32: 1461-1464.
24. Turton JF, Mustafa N, Shah J, Hampton CV, Pike R, Kenna DT. Identification of *Achromobacter xylosoxidans* by detection of the blaOXA-114-like gene intrinsic in this species. *Diagn Microbiol Infect Dis* 2011; 70:408–411.
25. Gajdács M. The concept of an ideal antibiotics: implications for drug design. *Molecules* 2019; 24: e892.