Isolation and Antibiotic Resistance of *Achromobacter xylosoxidans* from Non-respiratory Tract Clinical Samples: A 10-year Retrospective Study in a Tertiary-care Hospital in Hungary

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Author’s contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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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 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 (2008-2017) 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

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following: high levels of susceptibility for imipenem and meropenem (n=63; 92.6%), and moxifloxacin (n=55; 80.9%), while higher rates of resistance were detected for sulfamethoxazole/trimethoprim (susceptible: n=36; 52.9%), ciprofloxacin (susceptible: n=40; 58.8%) and almost all isolates were resistant to ceftazidime (susceptible: n=3; 4.4%) and cefepime (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.

ABBREVIATIONS

COL C: Colistin;
CZD C: Ceftazidime;
FEP C: Cefepime;
IMP I: Imipenem;
MER M: Meropenem;
CIP C: Ciprofloxacin;
MOX M: Moxifloxacin;
SXT S: Sulfamethoxazole/Trimethoprim;
CF C: Cystic Fibrosis;
MDR M: Multidrug-Resistant;
XDR X: Extensively Drug Resistant;
PDR P: Pandrug Resistant;
EUCAST E: European Committee for Antimicrobial Susceptibility Testing;
US U: United States;
HIV H: Human Immunodeficiency Virus;
ICU I: Intensive Care Unit;
CFU C: Colony-Forming Units;
MALDI-TOF MS: Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry;

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 [5], stool [6], urine [7], cerebrospinal fluid [8], peritoneal fluid [9], sputum [10], ear discharge [11], abscesses [12], bone and joint samples [13] and central venous catheters [10]. However, the most clinical data to date have been collected on A. xylosoxidans pneumonia in cystic fibrosis (CF) patients [14,15]. 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 [16]. 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 [17]. 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 [18].

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 [19]. 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 [20]. 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 [4-21]. 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) [10]. 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 [22]. Data
collection was performed electronically, based on
the following criteria: samples with significant
colonies counts for A. xylosoxidans (>10⁵ CFU/mL
for urine samples, while >10³ 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) [22]. 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
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
µL 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 [23].

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
µL 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 [24].

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 (CDZ), 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) [25]. Colistin susceptibility testing was not routinely performed, only per request of the clinicians. During data analysis, immediately-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 [26]. *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.).

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 CDZ (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* [26,27]. 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 [26-28]. 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 [29]. The reported mortality rate in invasive Achromobacter infections is around 2% for bacteremia, while this may reach 80% in case of neonatal sepsis [4-21,30].

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 [4-13,17,31,32]. 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 these drugs against Achromobacter species [32]. 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 (blaOXA-144-like, blaoXA-243), inducible (AmpC enzymes) and plasmid-mediated (blaphp) β-lactamases, and the effects of efflux pumps were also associated with β-lactam resistance [10]. In these cases, the therapeutic armamentarium for these infections narrows significantly [33,34].

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 in 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.

5. LIMITATIONS

Some limitations of this study should be noted: the retrospective design and the inability to access the medical records of the individual patients affected by these infections hindered the authors from assessing the correlation of the relevant risk factors and underlying pathologies with the isolation of A. xylosoxidans. The selection bias is a characteristic of such epidemiological studies, as most of these reports are originated from tertiary-care centers, corresponding to patients with more severe conditions or underlying illnesses. Lastly, the molecular characterization of resistance determinants in the mentioned isolates was not performed, non-susceptibility was characterized by phenotypic methods only. In future studies, a prospective study design and the comprehensive characterization of the medical history and laboratory parameters would aid the definition of the real pathogenic role of these bacteria.

DISCLAIMER

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

CONTENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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