ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology

www.iajlb.com
Email : editor@iajlb.com or iajlb.editor@gamil.com

Indo-Am. J. of LifeSc & Bt.,2020

ISSN 2347-2243 www.iajlb.com
Vol.17, Issue.2, May 2020

Indo-American Journal of Life Sciences and Biotechnology

Achromobacter xylosoxidans Isolation and Antibiotic Resistance from Non-respiratory Tract Clinical Samples: A Decade-Long Retrospective Study at a Tertiary Hospital in Hungary

Mr. Godlaveeti Balachandra, Mr. N. Dinesh, Mr. K. Nagasurendra,
Ms. Meruva Jyothi, Mrs. Syed Najma
Dr. C.H. Rajaharanadha babu

ABSTRACT

The purpose of this retrospective 10-year research was to determine the prevalence of A. xylosoxidans isolated from non-respiratory tract samples of adult inpatients and outpatients and the degrees of antibiotic resistance at a tertiary-care teaching hospital in Szeged, Hungary. The research strategy used electronic data collecting. Mueller-Hinton agar disk diffusion and, where necessary, E-tests were used to determine antimicrobial susceptibility.

The results showed that a total of 68 distinct A. xylosoxidans isolates were discovered during the course of the 10-year research period (6.83.6/year, range: 0-11 isolates). Isolation was more common in the second half of the research period (2013-2017) compared to the first half (2008-2017), when it was experienced by n=22. Fifty-one of the 68 isolates were from hospitalized patients. There were 32 female patients and 48 male patients, for a female-to-male ratio of 0.89. A. xylosoxidans isolates (n=68) showed similar susceptibilities.

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

Department of Pharmaceutical Analysis
Approved by AICTE& Pharmacy Council of India, New Delhi.(Affiliated to jawahalal Nehru Technological University. Anantapur&S.B.T.E.T.A.P)
Chennai-Hyderabad By Pass Road, Ukkayapalli, Kadapa-516002

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 clinicaldata to date have been collected on *A. xylosoxidans* pneumonia in cystic fibrosis (CF)patients [14,15]. The prevalence of this pathogenin 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 fortransplant rejection in the US, but the decreasein 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 infectionsin 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 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 clinicalmicrobiologists, 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 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. Theprocessing 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 werepassed 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 cellswere 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 (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) [25]. 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 [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 wereexcluded 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* [26,27]. The following virulence determinants have been identified inthe 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 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 plethoraof 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 ($bla_{OXA\text{-}144\text{-}like}$, $bla_{OXA\text{-}2}$, $bla_{OXA\text{-}243}$), inducible (AmpC enzymes) and plasmid-mediated (bla_{IMP}) 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 inthe 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.

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. Saiman L, Chen Y, Tabibi S, Gabriel PS, Zhou J, Liu Z, Lai L, Whittier S. Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. J Clin Microbiol 2011;39:3942-3945.
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.
5. Busse HJ, Stolz A. Achromobacter, alcaligenes and related Genera. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. (eds) The Prokaryotes. Springer, New York, NY; 2006.
6. Barragán EP, Pérez SJ, Corbella L, Orellana MÁ, Fernández-Ruíz M. Achromobacter xylosoxidans bacteremia: clinical and microbiological features in a 10-year case series. Rev Esp Quimioter. 2018;31:268-273.
7. Claassen SL, Reesen JM, Mysliwiec V, Mahlen SD. Achromobacter xylosoxidans Infection Presenting as a Pulmonary Nodule Mimicking Cancer. J Clin Microbiol. 2011;49:2751-2754.
8. Tena D, Gonzáles-Praetorius A, Pérez-Balsalobre M, Sancho O, Bisquert J. Urinary tract infection due to Achromobacter xylosoxidans: Report of 9 cases. Scand J Infect Dis. 2008;40:84-87.
9. Bellissimo F, Pinzone MR, Tosto S, Nunnari G, Cacopadro B. Achromobacter xylosoxidans meningitis in an immuno-suppressed patient. Int J Med 2014; 107:65-66.
10. First Documented Case of Percutaneous Endoscopic Gastrostomy (PEG) Tube-Associated Bacterial Peritonitis due to Achromobacter Species with Literature Review. Case Rep Gast Med; 2020. ID 4397930.
11. Krzewinski JW, Nguyen CD, Foster JM, Burns JL. 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.
12. Wiatr M, Morawska A, Skladzien J, Kedzierska J. *Alcaligenes xylosoxidans*--a pathogen of chronic ear infection. Otolaryngol Pol. 2005;59:277-280.
13. Asano K, Tada S, Matsumoto T, Miyase S, Kamio T, Sakurai K, Iida M. A novel bacterium Achromobacter xylosoxidans as a cause of liver abscess: Three case reports. J Hepatol. 2005;43:362-365.
14. Shinsha T, Oguagha IC. Osteomyelitis caused by Achromobacter xylosoxidans. IDCases. 2015;2:11-12.
15. 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.

16. 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.
17. 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.
18. Moissenet D, Baculard A, Valcin M, Marchand V, Tournier G, Garbarg-Chenon A, Vu-Thien H. 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.
19. 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.
20. 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