New Microbiologica, 46, 4, 412-415, 2023, ISSN 1121-7138

CASE REPORT

First case of *Chryseobacterium gallinarum* bloodstream infection: a diagnostic and therapeutic challenge for an emerging pathogen

Mattia Genco[1], Antonio Curtoni[1,2], Paolo Bottino[1], Silvia Scabini[3], Silvia Corcione[3], Davide Vita[3], Luisa Guarrasi[1], Gabriele Bianco[2], Alessandro Bondi[1,2], Daniela Risso[4], Rossana Cavallo[1,2], Cristina Costa[1,2]

[1]*Department of Public Health and Paediatrics, Microbiology and Virology Unit, University of Turin, Turin, Italy;*
[2]*Microbiology and Virology Unit, Molinette Hospital, City of Science and Health, Turin, Italy;*
[3]*Department of Medical Sciences, Infectious Diseases, University of Turin, Turin, Italy;*
[4]*Burn Center and Plastic Surgery, Department of General and Specialized Surgery, CTO Hospital, City of Science and Health, Turin, Italy*

SUMMARY

Chryseobacterium spp. belongs to the *Flavobacteriaceae* family and is a rod-shaped gram-negative, glucose non-fermenting, non-motile bacterium ubiquitous in the environment. In humans, *Chryseobacterium* may be responsible for infections such as urinary tract infections (UTI) and ventriculitis with a pathogenic burden increasing in recent years. *Chryseobacterium gallinarum* was isolated for the first time in 2014 in a pharyngeal scrape sample of chicken and, until now, only one case of human UTI has been described in a pregnant 20-year-old Indian patient. Herein, we report the first case of bloodstream infection caused by *C. gallinarum* in a 67-year-old female burn patient, correctly identified by 16S-rRNA sequencing and successfully treated with cefepime and fosfomycin.

Received October 19, 2023 *Accepted* December 01, 2023

INTRODUCTION

The genus *Chryseobacterium* belongs to the *Flavobacteriaceae* family, and was classified in 1994. *Chryseobacterium* are rod-shaped gram-negative, glucose non-fermenting, non-motile and ubiquitously distributed bacteria. They are usually inhabitants of soil and water, and their role as opportunistic pathogens has been increasing over the last few years: C. *gleum*, C. *indologenes*, and *C. arthorsphere* have been identified as aetiologic agents of urinary tract infections (UTI) and ventriculitis (Tai *et al.*, 2006; Shen *et al.*, 2005; Kim *et al.*, 2005; Machchhar *et al.*, 2023; Im *et al.*, 2020).
In 2014, Peter Kämpfer *et al.* isolated a new species of *Chryseobacterium* on nutrient agar from a pharyngeal scrape of a living and apparently healthy chicken in Saxony-Anhalt, Germany (Kämpfer *et al.*, 2014) They described smooth, yellowish, circular, and translucent colonies after 48 h of growth, and called them *Chryseobacterium gallinarum*. The whole genome of C. *gallinarum* was sequenced by Park *et al.* in 2015. Twenty-five open reading frames were identified as coding genes for virulence factors such as metallo- and serine-protease on a 4633,632 bp chromosome (Park *et al.*, 2015).
In 2022, at the University Hospital of Bhubaneswar, India, C. *gallinarum* was isolated for the first time in humans from the urine of a pregnant 20-year-old patient with UTI (Gaur *et al.*, 2022). The first identification performed by VITEK-II automated system (BioMérieux, Marcy L'Étoile, France) was C. *indologenes*, but subsequently, 16S-rRNA sequencing correctly identified it as C. *gallinarum*. Conventional phenotyping identification methods have very low accuracy in *Chryseobacterium* species differentiation: for this reason, molecular methods such as Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS) could be used, even if the gold standard 16S-rRNA sequencing should be preferred (Lin *et al.*, 2018).
Chryseobacterium spp. are intrinsically resistant to many antibiotics, including penicillin, carbapenems, aminoglycoside, and polymixin (*Table 1*) (EUCAST, 06-2023). The study by Mahendra Gaur *et al.* was the first to investigate the *C. gallinarum* antibiotic susceptibility profile: it was generally susceptible to fluroquinolones, sulphonamides, and tetracycline (Gaur *et al.*, 2022).
Herein, we report the first case of *C. gallinarum* blood-

Key words:
Bloodstream infection, emerging pathogen, *Chryseobacterium gallinarum*, *Chryseobacterium arthrosphaerae*, 16S-rRNA sequencing.

Corresponding author:
Antonio Curtoni
E-mail: antonio.curtoni@gmail.com

Table 1 - *Expected resistant phenotype of C. gallinarum based on document EUCAST Expected Resistant Phenotypes (v.1.2 January 2023) (EUCAST, 01-2023).*

Organism	*Ampicillin/Amoxicillin*	*Amoxicillin-clavulanic*	*Ampicillin-sulbactam*	*Ticarcillin*	*Ticarcillin-clavulanic acid*	*Piperacillin*	*Piperacillin-tazobactam*	*Cefotaxime/Ceftriaxone*	*Ceftazidime*	*Cefepime*	*Aztreonam*	*Ertapenem*	*Imipenem*	*Meropenem*	*Ciprofloxacin*	*Chloramphenicol*	*Aminoglycosides*	*Trimethoprim*	*Fosfomycin*	*Tetracyclines*	*Tigecycline*	*Polymyxin B/Colistin*
Chryseobacterium spp.	**R**	**R**	**R**	**R**	**R**			**R**	**R**		**R**	**R**	**R**	**R**			**R**					**R**

R: expected resistant phenotype.

stream infection (BSI) in a 67-year-old female patient with sepsis, correctly identified by 16S-rRNA sequencing and successfully treated with cefepime and fosfomycin.

CASE REPORT

In April 2023, a 67-year-old woman with a history of oligophrenia and hypothyroidism was admitted to the Burn Unit of the Traumatological Centre CTO, University Hospital Città della Salute e della Scienza, Turin, Italy. She presented wood stove burns, with a Total Body Surface Area (TBSA) involvement of 40%. Three days after admission she developed fever. An empirical antibiotic treatment with piperacillin/tazobactam and linezolid was started, and three blood culture sets were drawn simultaneously from the central venous catheter (CVC). Two aerobic bottles resulted positive after about 8 and 20 incubation hours in the absence of bacterial growth in anaerobic bottles. Gram staining showed Gram-negative bacilli, identified the next day on Columbia-blood-agar by Bruker Microflex® LT MALDI-TOF MS (Bruker Daltonics, Bremen) as *C. arthrosphere* with a *log* (score) >2.0 (*Figures 1* and *2*).

Antimicrobial susceptibility testing (AST) for cefepime, ciprofloxacin, trimethoprim-sulfamethoxazole, and tigecycline was performed with E-test (BioMérieux) and interpreted according to PK/PD EUCAST criteria v.13.0 (EUCAST, 01-2023).

The complete AST results and interpretation breakpoints are reported in *Table 2*. Cefepime was the only sensitive tested antibiotic with 0.5 minimum inhibitory concentration (MIC).

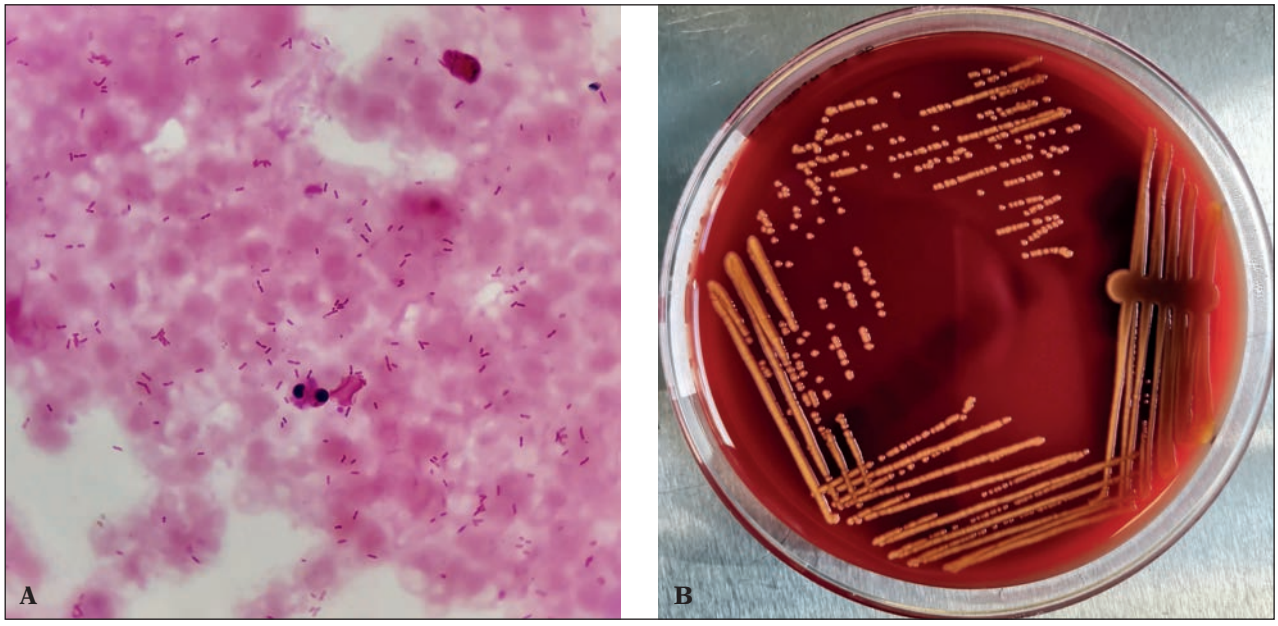

Figure 1 - *Chryseobacterium gallinarum Gram staining (A) and yellow and translucent colonies on Colombia-blood-agar (B).*

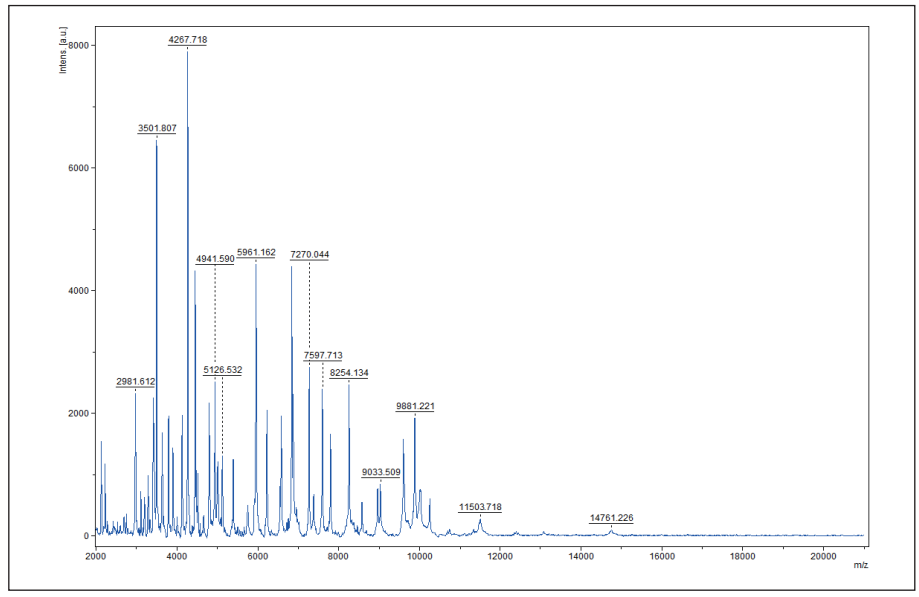

Figure 2 - *Chryseobacterium gallinarum MALDI-TOF MS spectrum.*

Table 2 - *Chryseobacterium gallinarum antimicrobial susceptibility profile.*

Antimicrobials	*MIC*	*Interpretation*	*MIC Breakpoint*	
			S≤	*R>*
Cefepime	0.5	S	4	8
Ciprofloxacin	0.38	I	0.25	0.5
Tigecycline	3	R	0.5	0.5
Trimethoprim-sulfamethoxazole	0.064	IE	–	–

I: susceptible, increased exposure; IE: insufficient evidence; R: resistant; S: susceptible, standard dosing regimen.

The CVC was replaced and the empirical antibiotic treatment was then switched to the combination therapy with cefepime 2 gr q8h, on the basis of AST results, and fosfomycin 8 gr q12h, according to the EUCAST expected resistant phenotype of *Chryseobacterium* and to fosfomycin potential synergic effect with cephalosporins in gram-negative non-fermenting bacteria bloodstream infections (Antonello *et al.*, 2020). Surgical incision of the burns (escharotomy) and a skin graft from the abdominal region were performed. After a 48-hour course, fosfomycin therapy was interrupted, whereas cefepime was continued until haemodynamic stability was reached and inflammatory markers showed improvement, completing 18 days of targeted antibiotic treatment.
Following this episode, the patient underwent several surgical incisions and skin grafts on the residual burns, the last of which 4 months after admission. She was then discharged home.
Given the rarity of the isolated species, especially as an aetiologic agent of human infections, 16S-rRNA sequencing was performed using the universal primers RU8 and U3. The sequence was aligned with reference sequences by GeneBank BLAST in NCBI public libraries and the strain was correctly identified as *C. gallinarum* (NIH, 2023).

DISCUSSION

In the last few years, *Chryseobacterium* has been isolated in various human samples [9]. Due to the elevated number of intrinsic resistances, in particular to β-lactams, these emerging pathogens could represent a growing public health problem, especially in immunocompromised patients. Furthermore, the clinical data on infection treatment and outcome are very limited.
We report the first case of *C. gallinarum* isolated from blood cultures in a burn patient with high TBSA. From a clinical perspective, although the rate of healthcare associated infections due to uncommon pathogens like *Chryseobacterium* spp. has gradually increased over the last decade, the real pathogenicity of these species is still unclear (Gaur *et al.*, 2022).
To the best of our knowledge, this is the first reported case of *C. gallinarum* BSI. The patient was successfully treated with fosfomycin and cefepime with no relapse of infection.
Conventional biochemical methods are not recommended for *Chryseobacterium* species differentiation. Furthermore, in this case the MALDI-TOF MS result, even with the highest score level, was not confirmed by sequencing. The use of the gold standard method

to identify emerging pathogens should be preferred. Unfortunately, especially for these bacteria, there is a limited number of reference sequences in NCBI public libraries.

Regarding *Chryseobacterium* infections, another pivotal problem is represented by the few available therapeutic options. On the one hand, *Chryseobacterium spp.* are intrinsically resistant to many antimicrobial classes; on the other, few antibiotics could be reported due to the absence of EUCAST/CLSI interpretation breakpoints and ECOFFs.

In conclusion, *Chryseobacterium,* especially *C. gallinarum*, represents an important diagnostic and therapeutic challenge for both identification and AST issues.

We advocate for studies to improve *Chryseobacterium* species differentiation, with the acquisition of new specific MALDI-TOF MS spectra and genome sequences. Moreover, whole genome sequencing studies could deepen antimicrobial genes characterization, thus improving the clinical-therapeutic management of these infections.

Due to these obstacles, the evidence and impact of *C. gallinarum* in human infections may be underestimated: this case report helps to highlight these issues and to provide useful information for *C. gallinarum* identification and treatment.

Acknowledgements

We would like to acknowledge the personnel of the Burn Center and Plastic Surgery (CTO Hospital) and of the Bloodstream Infection Section of the Microbiology and Virology Unit (Molinette Hospital) for their valuable contribution.

Funding information

This research was supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

Author contributions

M.G. and A.C. wrote the paper; P.B., S.S., S.C., D.V, L.G., G.B., A.B., D.R., R.C. and C.C. revised the paper; D.R., S.S., S.C. and D.V. had the patient in charge; A.C. and A.B. supervised the work; C.C. coordinated the work.

Abbreviations

AST: antimicrobial susceptibility testing; Bp: base pair; BSI: bloodstream infection; CLSI: Clinical and Laboratory Standards Institute; CVC: central venous catheter; ECOFF: epidemiological cut-off

EUCAST: European Committee on Antimicrobial Susceptibility Testing; MALDI-TOF: Matrix-Assisted Laser Desorption/Ionization Time of Flight; MIC: minimum inhibitory concentration

MS: Mass Spectrometry; PK/PD: pharmacokinetic/pharmacodynamic; TBSA: Total Body Surface Area; UTI: urinary tract infections

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki. Written informed consent to anonymous publication was obtained from the patient.

References

Antonello R.M., Principe L., Maraolo A.E., Viaggi V., Pol R., Fabbiani M., et al. (2020). Fosfomycin as Partner Drug for Systemic Infection Management. A Systematic Review of Its Synergistic Properties from In Vitro and In Vivo Studies. *Antibiotics* (Basel). **10**, 9 (8): 500. doi: 10.3390/antibiotics9080500. PMID: 32785114; PMCID: PMC7460049.

EUCAST, Clinical Breakpoints Tables version 13.1, June 2023.

EUCAST, Expected Resistant Phenotypes version 1.2, January 2023.

Gaur M., Dey S., Sahu A., Dixit S., Sarathbabu S., et al. (2022). Characterization and Comparative Genomic Analysis of a Highly Colistin-Resistant *Chryseobacterium gallinarum*: a Rare, Uncommon Pathogen. *Front Cell Infect Microbiol.* **12**, 933006. doi: 10.3389/fcimb.2022.933006. PMID: 35909954; PMCID: PMC9329510.

Im J.H., Kim D., Kim J.J., Kim E.Y., Park Y.K., et al. (2020). *Chryseobacterium arthrosphaerae* ventriculitis: A case report. *Medicine* (Baltimore). **99** (34), e21751. doi: 10.1097/MD.0000000000021751. PMID: 32846799; PMCID: PMC7447447.

Kämpfer P., Poppel M.T., Wilharm G., Busse H.J., McInroy J.A., et al. (2014). *Chryseobacterium gallinarum sp. nov.*, isolated from a chicken, and *Chryseobacterium contaminans sp. nov.*, isolated as a contaminant from a rhizosphere sample. *Int J Syst Evol Microbiol.* **64** (Pt 4), 1419-1427. doi: 10.1099/ijs.0.058933-0. Epub 2014 Jan 21. PMID: 24449786.

Kim K.K., Bae H.S., Schumann P., Lee S.T. (2005). *Chryseobacterium daecheongense sp. nov.*, isolated from freshwater lake sediment. *Int J Syst Evol Microbiol.* **55** (Pt 1), 133-138. doi: 10.1099/ijs.0.02931-0. PMID: 15653865.

Lin J.N., Teng S.H., Lai C.H., Yang C.H., Huang Y.H., et al. (2018). Comparison of the Vitek MS and Bruker Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Systems for Identification of *Chryseobacterium* Isolates from Clinical Specimens and Report of Uncommon *Chryseobacterium* Infections in Humans. *J Clin Microbiol.* **56** (11), e00712-18. doi: 10.1128/JCM.00712-18. PMID: 30135228; PMCID: PMC6204688.

Machchhar .RR., Yang J.S., Figueroa M., Ghodasara K., Hasan B. (2023). A Curious Case of *Chryseobacterium indologenes* Culture in a Young Adult Kidney Transplant Patient. *Cureus.* **15** (1), e33395. doi: 10.7759/cureus.33395. PMID: 36751170; PMCID: PMC9899075.

NIH, https://blast.ncbi.nlm.nih.gov/blast.cgi.

Park G.S., Hong S.J., Jung B.K., Khan A.R., Park Y.J., et al. (2015). Complete genome sequence of a keratin-degrading bacterium *Chryseobacterium gallinarum* strain DSM 27622(T) isolated from chicken. *J Biotechnol.* **211**, 66-67. doi: 10.1016/j.jbiotec.2015.07.007. Epub 2015 Jul 21. PMID: 26209507.

Shen F.T., Kämpfer P,. Young C.C., Lai W.A., (2005). Arun AB. *Chryseobacterium taichungense sp. nov.*, isolated from contaminated soil. *Int J Syst Evol Microbiol.* **55** (Pt 3), 1301-1304. doi: 10.1099/ijs.0.63514-0. PMID: 15879271.

Tai C.J., Kuo H.P., Lee F.L., Chen H.K., Yokota A., et al. (2006). *Chryseobacterium taiwanense sp. nov.*, isolated from soil in Taiwan. *Int J Syst Evol Microbiol.* **56** (Pt 8), 1771-1776. doi: 10.1099/ijs.0.64294-0. PMID: 16902006.