Chapter 12
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Moellerella wisconsensis, a Review and Case Study on Rarely Isolated Organism from Clinical and Non-clinical Specimens

Anurag Zaveri[1*] and Lakshmi B.[2]

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ABSTRACT

Isolation and reporting of rarely found and opportunistic pathogens in the gastro-intestinal tract is not observed in medical fraternity because of many reasons. Primarily, it could be because of abundant presence of normal flora, hence the presence of rare organisms might be out looked in routine practice. Secondarily, it could be due to the limited knowledge and resources to isolate and report such findings in daily busy practice. Lastly, it could be because of its non-frequent occurrence. Nonetheless, cases of isolating the organism *Moellerella wisconsensis* have been reported since its first report published in 1984, by Hickman Brenner. The chapter discusses the history and reports *Moellerella wisconsensis* from a stool sample for the first time in the western region of India (Ahmedabad, Gujarat) along with other reported incidences from various clinical and non-clinical sources [1,2,3,4,5,6,7,8].

Keywords: Moellerella wisconsensis; diarrhea; stool; enteric bacteria; pathogen.

1. HISTORY AND INTRODUCTION

Genus of the organism, *Moellerella wisconsensis*, previously included in the Enteric Group 46, was first isolated by Hickman Brenner in the year 1984, where genus was named after a Danish microbiologist Vagn Moeller, species was named after Wisconsin, a state in America, from where the highest number of strains (six out of nine) were isolated. The first organism had been isolated from stool sample (CDC# 2252-80) of a patient (a physician visiting Peru) from the USA. His stool samples showed scanty growth of *Escherichia coli* and no pathogenic flora, viz. *Salmonella spp.*, *Shigella spp.*, *Yersinia spp.*, *Campylobacter spp.*, or *Aeromonas spp.* Successive specimen received by CDC did not show the presence of pathogenic organisms like *Salmonella spp.*, or *Shigella spp.*; however, *Yersinia spp.* was isolated from the specimen from a 29 year old male with diarrhea (CDC 2397-81). Isolate# 3065-75 and 1826-79 were obtained from patients with active diarrhea; whereas, from a patient having gastroenteritis isolate# 2552-77 was obtained with no further information [1].

Out of total nine strains of *Moellerella wisconsensis* studied from samples of different origin, eight strains were isolated from stool samples; whereas, single strain was isolated from drinking water source from South Dakota which did not chlorinate its water [1]. Since then presence of the strain in various clinical and non-clinical sources has been observed. Various case studies have been published reporting isolation of the organism from human specimen like gall bladder tissue [3,4], bile [5], bronchial aspirate [6] and blood [7,8] as well as non-clinical specimens like food and water [2,9,10,11,12,13], and samples of animal origin like liver and kidney tissue of a cow [14].

While studying the organism in detail for the first time, morphological characteristics and results of various biochemical tests were compared with that of other known organisms along with the results of

[1]*Biocare Research (India) Pvt. Ltd., India.*
[2]*Department of Biotechnology and Microbiology, SMMPISR, Kadi Sarva Vishwavidyalaya, Gandhinagar, India.*
**Corresponding author: E-mail: anuragzaveri@yahoo.com;*

the DNA relatedness study. *Moellerella wisconsensis* was found to be Gram-negative, rod-shaped and facultative anaerobic organism [15]. The biochemical test results revealed that the organism was showing positive results to the tests like Methyl red, citrate, KCN (growth in potassium cyanide broth), glucose degradation, and various acid hydrolysis tests along with nitrate reduction and OPNG (lactose fermentation test) which were found to be the same for ATCC 35017 strain. DNA relatedness of five strains of *Moellerella wisconsensis* was compared with the ATCC strain at 60°C and 75°C and the relatedness was found to be more than 80 percent for wild type strains with standard strain. DNA relatedness study between *Moellerella wisconsensis*' and other organisms' genomic DNA revealed that *Moellerella wisconsensis* has 32 percent DNA similarity with *Providentia rustigianii* 26240 (formerly known as *Providentia alcalifaciens*) at 60°C, but the relatedness decreased to 4-7 percent at more stringent 75°C incubation temperature. Antibiotic susceptibility results studied by Hickman-Brenner of all nine isolates revealed that all the strains isolated were resistant to colistin at 10µg concentration; whereas, variable susceptibility was observed in other antibiotics (nalidixic acid, sulfadiazine, gentamycin, streptomycin, kanamycin, tetracycline chloramphenicol, ampicillin carbenicillin and cephalothin) at different drug concentrations [1].

2. RECENT ADVANCEMENTS

Since its first publication, there have been quite a few incidences of isolating *Moellerella wisconsensis* from various sources other than specimen of human origin. With the emergence of sophisticated technologies and previous mentions, it was not absurd to report such rarely isolated organisms. Last report on isolation of *Moellerella wisconsensis* was published by a researcher and her colleagues from Vasco De Gama University, Portugal, having isolated the organism from liver and kidney tissues of a cow, making it the fifth isolation of the organism from non-clinical sources [14].

The author of this chapter reported a case study on isolating *Moellerella wisconsensis* for the first time in India from stool sample in the year 2018 providing insight to other researchers to explore. In January 2020, second case has been published reporting co-existence of bla_{NDM-1} and bla_{VIM-1} genes in *Moellerella wisconsensis* in India [16]. The case reports raise serious question against the upcoming global threat of antibiotic resistance, the organism which was resistant only to colistin is now showing resistance to all other antibiotics leaving clinicians with no option.

3. CASE REPORT

This case study reports the presence of *Moellerella wisconsensis* from a stool sample of a 75 years old bed ridden lady having prolonged history of diarrhea admitted to a tertiary care hospital of Ahmedabad. The lady had a chief complaint of vomiting, pressure sore, diarrhea with previous history of fracture of neck. At the time of admission, her pulse was 118/minute and blood pressure was 100/60 mm of Hg along with tenderness in epigastrium, fever was absent though.

4. RESULTS AND DISCUSSION

Treatment, from the beginning, was centered focused to cure prolonged diarrhea. Treating physicians were doubting infection of *Clostridium difficile*, acid peptic disease and stress induced diarrhea. Laboratory findings like potassium level (2.90mmol/L), sodium level (131.00mmol/L), total protein (5.80), A/G ratio (1.00), Albumin (2.90), Blood Gas Analysis pCo (2-26mm of Hg) HCO3 (19.80mmol/L) and Base excess (2.9) were found to be within normal biological range. Urine and stool samples were sent to the laboratory for culture and susceptibility analysis and *E. coli* was identified as pathogen isolated from urine sample using API®/ID32 card used for identification of Gram negative bacilli. The isolate of *E. coli* was sensitive to carbapenem, aminoglycosides, tetracyclines and nitrofurantoin.

Stool culture showed presence of mix flora on MacConkey agar medium after 24 hours of incubation. The isolated organisms were processed individually for identification through semi-automated identification system Mini-API (by Biomerieux, France) using API®/ID32 card. Lactose fermenting isolate was identified as *E. coli* by the machine; whereas, lactose non-fermenting isolate was

identified as *Moellerella wisconsensis*. Using differential medium, colonies of both the organisms were separated and re-checking was carried out for the isolates and results received at the end of the second identification test were found to be the same as earlier along with other biochemical tests run manually.

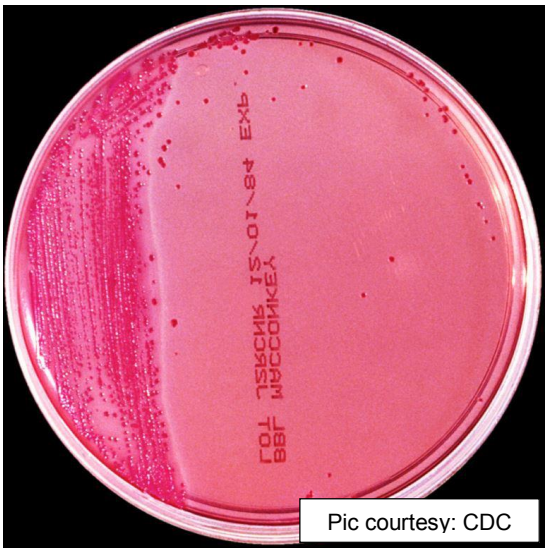

Fig. 1. Growth of *Moellerella wisconsensis* after 24 hours of incubation on MacConkey agar plate [15]

The tests previously reported matched with the manual biochemical tests and the semi-automated system, for the second time, identified the organism as *Moellerella wisconsensis* with 97 percent match with no test against the profile. Antibiotic susceptibility testing for *Moellerella wisconsensis* isolate was found to be matching with the classic paper on identification of the novel organism as *Moellerella wisconsensis.* The isolate was susceptible to ampicillin+sulbactum, piperacillin+tazobactum, imipenem, meropenem, ertapenem, doripenem, amikacin, netilmycin, chloramphenicol, tetracycline, dosycycline, minocycline, and tigecycline and the isolate showed resistance towards colistin. Similar antibiotic susceptibility findings were obtained in a study on *Moellerella wisconsensis* isolated from samples of human, food and water origin [11].

Moellerella wisconsensis whole genome sequencing was carried out as a part of Public Health England NCTC reference collection new eResource. The data generated through this project, carried out in collaboration with Sanger Institute and Wellcome Trust, will be hosted in public domain [17]. Further DNA analysis could not be carried out for reported case due to financial and infrastructure limitations.

The patient was treated with imperial dosages of injectables and started recovering from the fourth day of treatment. The patient was discharged after 5 days of antibiotic treatment with stable vitals [2].

5. CONCLUSION

Finding opportunistic and rare pathogens in normal flora rich samples, stool for an instance, is a challenge. Reporting the same is bigger challenge due to rarer publication footprints in the fraternity. Presence of such organism could normally be overlooked by technicians due to the abundant presence of normal flora and such organisms could be easily left unreported. The reservoir of such organism should be another concern as the organism has been isolated from all possible reservoirs, water, food, animals and humans. Pathogenicity and virulence of *Moellerella wisconsensis* is highly concerned issue and should be taken into consideration while treating patients with such infections

[18]. A recently published article reported presence two popular carbapenem genes, VIM and NDM in *Moellerella wisconsensis* raising concerns of treating physicians [16].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Biography of author(s)

Anurag Zaveri
Biocare Research (India) Pvt. Ltd., India.

Research and Academic Experience: 06 years.

Research Area: Antimicrobial Resistance, Medical Microbiology, Biotechnology, medical devices.

Number of Published papers: 10.

Citations: 07.

Patent/s: 02 (Medical Devices).

Any other remarkable point(s): The author is one of the 30 selected scientists from India, who are working on antimicrobial resistance, whose strains are submitted for whole genome sequencing data of which will ultimately be stored in NHS whole genome repository. The project is jointly funded by Bill and Melinda Gates Foundation and Wellcome Trust, UK in technical association with Sanger Institute. Apart from that the author is AMR ambassador for AMR Insights, having its HQ in The Netherlands.

Dr. Lakshmi B.
Department of Biotechnology and Microbiology, SMMPISR, Kadi Sarva Vishwavidyalaya, Gandhinagar, India.

Research and Academic Experience: 15 years of Academic and 12 years of research experience.

Research Area: Cancer biology, Bioremediation, Microbial enzymology and Antibiotic resistance.

Number of Published papers: 26 published papers and two accepted.

Any other remarkable point(s): The author is one of the 30 selected scientists from India, who are working on antimicrobial resistance, whose strains are submitted for whole genome sequencing data of which will ultimately be stored in NHS whole genome repository. The project is jointly funded by Bill and Melinda Gates Foundation and Wellcome Trust, UK in technical association with Sanger Institute. Apart from that the author is AMR ambassador for AMR Insights, having its HQ in The Netherlands.