Non-fermenting Gram-negative bacilli (NFGNB) other than *Pseudomonas*

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INTRODUCTION

Non-fermenters are a heterogenous group of Gram-negative bacilli that are aerobic, non-sporing, either do not use carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. Being ubiquitous in nature, they were disregarded as probable contaminants, when isolated in the laboratory. However, they have now emerged as important healthcare associated pathogens as they have made their niche in the hospital environment. Emerging challenges of multi-drug resistance, both intrinsic and acquired among them is of serious concern to the treating physician.[1]

Non-fermenters account for 15% of all bacterial isolates from a clinical microbiology laboratory. Published studies from various centres quote varied isolation rates of non-fermenters ranging from 2.18% to 45.9%.[2]

Taxonomic confusion prevails as there is a continuous revision and many of the identified strains have no designated species assigned.[3] This is compounded by the factors that contribute to the difficulties in identifying them in the routine clinical microbiology laboratory. Most species are infrequently encountered and therefore, the laboratory personnel may not be familiar with many of the non-fermenters. Many of the conventional culture media are not suitable for identification and quality control of media may be difficult. Many species are slow growing and biochemically weak or inert and requires considerable experience to interpret equivocal results. Commercial kit systems that are available for use are not only expensive but also often have low accuracy for identification of certain strains.[4]

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Most clinical microbiology laboratories rely mainly on the phenotypic methods of identification. These may include manual or commercial kit/automated identification systems, such as API 20NE, Remel N/F, Vitek 2, Microscan Walkaway, the Sensititre AP80 system, the Phoenix system.[4]

However, studies investigating the performance of the commercial identification system have shown contradictory results. Identification by conventional phenotypic methods can be difficult and time consuming. Molecular identification techniques are emerging as alternate for phenotypic identification methods. Among these are 16s rRNA gene sequencing and DNA array (oligonucleotide array) technique that have been described as reliable and rapid method for identification of clinically significant Non-fermenting Gram negative bacilli (NFGNB).[5]

The list of NFGNB is endless and beyond the scope of this article. Few commonly encountered clinically important non fermenters other than *Pseudomonas* are high lighted in this article.

GENUS ACINETOBACTER

As evidenced by our data in the special article in this issue, and most other studies, the most common non-*Pseudomonas* non-fermenter recovered from clinical specimens is Acinetobacter. Classified under the family Moraxellaceae, this genus includes Gram-negative coccobacilli that are non-motile, oxidase negative and resistant to penicillin.[4] More than 25 genomospecies have been recognised by DNA–DNA hybridisation within the genus and seven have been given formal species name. Among these are species *Acinetobacter calcoaceticus, A. baumannii*, *Acinetobacter* genomic species 3 and *Acinetobacter* genomic species 13TU that have an extremely close relationship and are difficult to distinguish from each other by phenotypic tests alone. They have been

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grouped as *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex.[6]

A. baumannii is saccharolytic, acidifies most carbohydrates and demonstrates the rapid production of acid from 1% and 10% lactose. These features can be used for their presumptive identification in the routine diagnostic laboratory. *A. baumannii* complex accounts for 80% of clinical infections caused by Acinetobacter species, and includes pneumonia, bacteraemia, meningitis, urinary tract infection (UTI) and wound infections, most of which are hospital-acquired.[4,6]

Acinetobacters have emerged as the most successful pathogens by their ability to survive and persist in the hospital environment for extended time periods both in dry and moist surfaces. This is assisted by their ability to grow at a range of different temperatures and pH, thereby contributing to the development and persistence of outbreaks.[6] Compounding this problem is their ability to produce biofilms on the surface of medical devices.[7]

Multiple resistance mechanisms are found in this organism that have contributed to the emergence of multidrug and pan drug resistance, causing a serious concern for the treating physician.[6,8]

Interestingly, in a study of an epidemic multidrug-resistant Acinetobacter strain in France, a large genomic resistant island containing 45 resistant genes that have been acquired from other Gram-negative bacilli has been reported.[9] Antibiotic susceptibility testing for Acinetobacter species is problem-prone and results obtained by using standardised microbroth dilution do not agree with that obtained by standard disk diffusion method particularly for beta lactam and beta lactam inhibitor combination.[4] The clinical and laboratory standards institute (CLSI) does not define the guidelines for disk diffusion testing and interpretation for newer antibiotics like Tigecycline and Colistin.[10]

GENUS BURKHOLDERIA

Burkholderia cepacia (B.cepacia)

A phytopathogen, *B. cepacia* has emerged as a cause of opportunistic infection particularly in patients with chronic granulomatous disease and cystic fibrosis.[4]

Taxonomic studies have demonstrated that *B. cepacia* is actually a cluster of at least nine closely related genomovars. They are most frequently associated with epidemics of 'Cepacia syndrome' manifested by severe progressive respiratory failure and bacteraemia. *B. cepacia* complex has been isolated from numerous water sources and wet surfaces including detergent solutions and IV fluids. Hospital out break due to common source contamination of medical devices such as nebulizers, disinfectants and blood gas analyzers has been reported.[3]

Identification of *B. cepacia* in the clinical laboratory may be problematic because it is not a single phenotype. Commercial identification systems perform poorly.[4]

Primary culture from clinical specimens may be performed on selective media such as *B. cepacia* selective agar or oxidation fermentation polymyxin bacitricin lactose agar (OFPBL agar) incubated at 35°C for 48 hours. Colonies appear yellow due to lactose utilisation. The isolate is weakly oxidase positive, hydrolyses lysine and is resistant to Polymyxin B and aminoglycosides but sensitive to Co-trimoxazole.[4]

Treatment of choice is Co-trimoxazole. CLSI suggests *in vitro* testing for Ceftazidime, Meropenem, Minocycline (tetracycline) and Co-trimoxazole.[10]

Burkholderia pseudomallei (B.pseudomallei)

A causative agent of Melioidosis, *B. pseudomallei* is a Hazard Group 3 pathogen and the safety of the laboratory worker is of prime concern while handling this organism.[3]

B. pseudomallei should be considered in patients with pneumonia, sepsis or abscess with a travel history to South-East Asia or northern Australia. The organism is not difficult to isolate in routine media. However, variation in colony morphology may be seen. It can grow at 42°C. Gram-stained smears from clinical specimens show a bipolar staining pattern. Isolation of the organism from non-sterile sites requires the use of a selective medium, the Ashdown's medium, which shows rough wrinkled violet or purple colonies after 48 hours. An oxidase positive motile Gram negative bacilli that, can be identified by its characteristic antibiogram showing constitutive resistance to Polymyxin and Gentamicin but sensitive to Co-amoxyclavulanic acid, Tetracycline and Chloramphenicol. Commercial kits identify well, of which API 20NE is the best validated one.[3]

Stenotrophomonas maltophilia

It is the third most commonly encountered non-fermenter in clinical practice. Being ubiquitous in nature, it can colonise the respiratory tract in hospitalised patients and cause nosocomial infections such as CRBSI (catheter associated blood stream

infections), and pneumonia particularly in patients with haematological malignancy.

It produces pale yellow to lavender green colonies on blood agar. It is an oxidase negative, motile bacillus that is characteristically resistant to Imipenem (Carbapenem), but sensitive to Colistin, Polymyxin, Cotrimoxazole, Minocycline and Levofloxacin. It is a strong maltose oxidiser being lysine and DNAase positive. Most commercial kits are able to identify this organism.[3]

However, one should exercise caution while reading and interpreting the antibiotic sensitivity tests. Trailing end points have been observed in agar dilution and broth dilution tests and falsely sensitive readings with disk diffusion assay have occurred for Gentamicin and Ciprofloxacin. Similarly, studies using E test have noted the presence of tiny micro colonies or a haze of translucent growth within the area of inhibition, which if missed could lead to falsely sensitive result.[4]

CLSI suggests the following antibiotics to be tested for Stenotrophomonas maltophilia. Cotrimoxazole (drug of choice), Ceftazidime, Levofloxacin, Minocycline, Chloramphenicol and Ticarcillin. MIC by broth dilution is recommended for testing Ceftazidime, Chloramphenicol and Ticarcillin, as the disk diffusion method is unreliable.[10]

Chryseobacterium meningosepticum (Elizabethkingia meningosepticum)

Although rare, it is important to identify this organism as it may cause outbreaks in nursery units and is associated with high mortality rates (50%). A soil saprophyte, it can contaminate patient care articles resulting in neonatal meningitis or sepsis.

The organism produces pale yellow pigmented colonies on blood agar, which may take more than 24 hours to grow. It is a non-motile, Gram-negative rod, that is, oxidase positive, indole positive, hydrolyses aesculin and gelatin and demonstrates a positive ONPG test. The isolate is susceptible to Penicillin, Vancomycin, Cotrimoxazole and Fluoroquinolones.[3,4]

This organism has two types of beta-lactamases: extended spectrum beta lacatamases (ESBL) and metallo beta lactamases (MBLs) that confer resistance to Cephalosporins and Carbapenems. Therefore antibiotics used in the treatment of Gram-negative infection cannot be used for treating Chryseobacterium infections. MICs for Vancomycin on clinically significant isolates needs to be performed. Disk diffusion tests are unreliable.[11]

CONCLUSION

All clinical microbiology laboratories must be geared to accurately identify the non-fermenters and clinical significance of an isolate must be determined on a case by case basis. Precise identification is important for optimal patient management, prognosis and appropriate infection control intervention. The type of identification system used by the laboratory should be left to the discretion of the clinical microbiologist. However, it is essential to ensure that the quality and the performance of the systems are validated periodically.

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