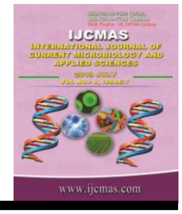
Int.J.Curr.Microbiol.App.Sci (2019) *8*(7): 1851-1858

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 07 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.807.220

Prevalence of Non - Fermenters among Various Clinical Samples and their Antibiotic Resistance at Tertiary Care Centre Jhalawar, India

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Keywords

NFGNB, Multidrug resistance, Cross-sectional study

Article Info

Accepted:
15 June 2019
Available Online:
10 July 2019

A B S T R A C T

Accurate identification, isolation and antibiotic sensitivity pattern of non-fermenting gram-negative bacilli (NFGNB) is important for proper patient management. Aim: to find out the prevalence and the antibiotic sensitivity pattern of non- fermenters (NFs). A cross-sectional study was conducted on specimen collected from patients of tertiary care centre and send to microbiology laboratory for culture and sensitivity. Out of 4819 specimens collected in a year, 3363 (69.78%) were found culture positive and among them 378 (7.84%) were positive for NFGNB. Maximum percentage of NFs was from surgical wards (38.88%) followed by medical ICUs (24.07%) and surgical ICUs (21.69%). *P. aeruginosa* was the predominant isolate (47.88%) followed by *A.baumannii* (38.09%). Pus (37.83%) was the most common specimen shows growth of NFs followed by urine (16.93%), wound swab (16.40%), sputum (8.73%) and blood (8.20%). The antibacterial sensitivity pattern of NFGNB showed that isolated organisms were susceptible to higher generation of antibiotics, but showed some degree of resistance to routinely used drugs. NFGNB which was thought to be commensals or contaminants are now emerging as important nosocomial pathogens and keeping in mind the antibiotic sensitivity pattern, judicial use of antibiotics is required for management of concern infection.

Introduction

Taxonomically, Non-fermenting Gram-negative bacilli (NFGNB) are a diverse group of organisms that either do not use carbohydrates as a source of energy or degrade them through metabolic pathway other than fermentation.[1,2,3] The pathogenic potential of NFGNB has been established beyond doubt because of their repeated isolation from clinical specimens especially in hospitalized patients and association with the disease. These diverse groups of microorganisms have recognizable traits of clinical importance to justify their inclusion in a single group. They have been commonly found on the body of healthcare workers, instruments used in hospitals such as ventilator machines, humidifiers and mattresses and are known to account for nearly 12%–16% of all bacterial isolates from a clinical laboratory. [4,5]

Accurate identification, isolation and antibiotic sensitivity pattern of non-fermenters is important for proper patient management. Infection caused by non-fermenters can be endogenous or exogenous in origin, which depends on several factors such as: irrational use of antibiotics, unjustified use of immune-suppressants, lengthened surgical procedure and inadequate instrumentation.[6] Infections usually caused by these bacteria are septicemia, urinary tract infections, meningitis, pneumonia and surgical site infections.

Most commonly isolated NFGNB are Pseudomonas aeruginosa and *Acinetobacter baumannii*.[7]

Multidrug resistance is common and increasing amongst NFGNB and a number of strains have now been identified that exhibit resistance to essentially all commonly used antibiotics by several mechanisms like antimicrobial inactivating enzymes, reduced access to bacterial targets and point mutations that change targets or cellular functions.[8,9]

Such antibiotics resistance compromises treatment, prolongs hospital stay, increases mortality and healthcare costs.[10,11] In view of these facts current study was undertaken to find out the prevalence as well as the antibiotic sensitivity pattern of NFGNB in various clinical samples.

The main objectives of this study, to determine the prevalence of NFGNB isolated from various clinical specimens. And to evaluate antimicrobial susceptibility profiles of NFGNB.

Materials and Methods

A cross-sectional study was conducted on clinical specimens collected from patient attending OPD or IPD of Jhalawar Medical College, Jhalawar during 1st July 2018 to 30th June 2019. A total 4819 clinical specimens including blood, urine, pus, CSF, sputum, body fluids, wound swab etc were received in laboratory for microbiological assessment and antibiotic susceptibility. Baseline data such as age, gender, type of specimen etc was assessed from investigation requisition slip and filled in predesigned proforma. All collected specimen was inoculated in appropriate media (MacConkey agar, Nutrient agar and Blood agar) and incubated aerobically at 37°C for 18 to 24 hours. Isolation and identification was based on the Gram staning, motility, biochemical test and colony morphology on culture media. Specimens suspected positive for non- fermenters were further identified using Biomerieux vitek-2 system.[12]

Antimicrobial susceptibility was performed for all confirm isolates by modified Kirby - Bauer disc diffusion method. Interpretations were made using the Clinical and Laboratory Standards Institute, USA guidelines.[13]

The different antimicrobials used were gentamicin (10µg), amikacin (30 µg), ceftazidime (30µg), ceftriaxone (30µg), piperacillin/tazobactum (100µg/10µg), imipenem (10µg), meropenem (10µg), ciprofloxacin (5µg), and cotrimoxazole (25µg) etc. *Escherichia coli* ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control strains.[13]

Statistical analysis

Data was entered in Microsoft excel 10 and SPSS 24.0 for analysis. Data was presented in tables, graph and charts. Descriptive statistics were used. P values <0.05 was considered as significant.

Ethical consideration

Study was started after taking ethical approval from institutional ethic committee.

Results and Discussion

A total 4819 clinical specimens were received for microbiological assessment and antibiotic susceptibility in a year.

Out of 4819 specimens, 3363 (69.78%) were found culture positive and among them 378 (7.84%) were positive for NFGNB (Figure 1).

Majorities (49.73%) of patients were adults aged above 45 years and isolation rate of NFs was higher in males (59.25%) in comparison to females (40.75%).

Maximum percentage of NFs was from surgical wards (38.88%) followed by medical ICUs (24.07%) and surgical ICUs (21.69%).

P. aeruginosa was the predominant isolate (47.88%) followed by *A. baumannii* (38.09%) and *A. lwoffi* (5.8%) while *B. cepacia*, *S. maltophilia* and *P. stutzeri* altogether accounted for 8.20% (Figure 2 and Table 1).

Pus (37.83%) was the most common specimen shows growth of NFs followed by urine (16.93%), wound swab (16.40%), sputum (8.73%) and blood (8.20%). Body fluids were 6.87%, including ascetic fluid, pleural fluid, CSF etc. Other specimens were collected from catheter tip, drain tip and swab from respiratory tract or reproductive tract. Among NFs, *P.aeruginosa* and *A.baumannii* was found more commonly in pus, urine and wound swab although *A.lwoffi* was predominantly found in urine and *B.cepacia* in sputum (Table 1).

The antibacterial sensitivity pattern of NFGNB as a pathogen showed that isolated organisms were susceptible to higher generation of antibiotics, but showed some degree of resistance to routinely used drugs like ciprofloxacin, gentamicin amikacin, and Co-trimoxazole. Most common isolate *P. aeruginosa* is sensitive to Imipenem (84.53%), Meropenem (85.08%), Piperacillin/ Tazobactum (82.32%) and Polymyxin -B (100%) while sensitivity to Ciprofloxacin and Gentamicin was below 50%.

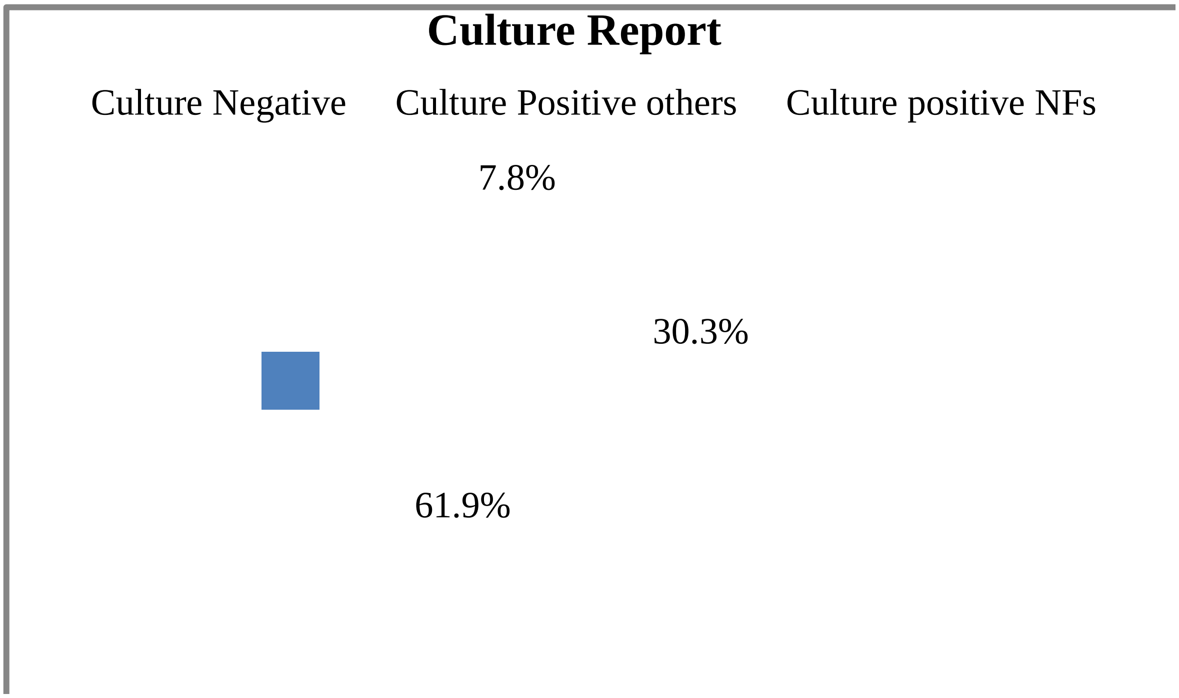
Sensitivity of second most isolated NFGNB, *A.baumannii* is near 50% for Ciprofloxacin, Gentamicin and Cotrimoxazole while it is above 70% for Imipenem (78.47%), Meropenem (80.56%), Piperacillin/ Tazobactum (77.08%) and Polymyxin –B (100%).

Lowest sensitivity in *A.lwoffi*, *B.cepacia*, *S.maltophilia*, *P.stutzeri* was found for Gentamicin (50%), Ciprofloxacin (37.5%), Piperacillin/Tazobactum (44.5%) and Gentamicin (50%) respectively while most of NFs were sensitive for Polymyxin –B (100%) (Table 2).

NFGNB are ubiquitous in environment. They used to be considered as commensals or contaminants in the past. They have now emerged as important healthcare-associated and opportunistic pathogens due to their frequent isolation from clinical specimens and their association with various diseases.[14]

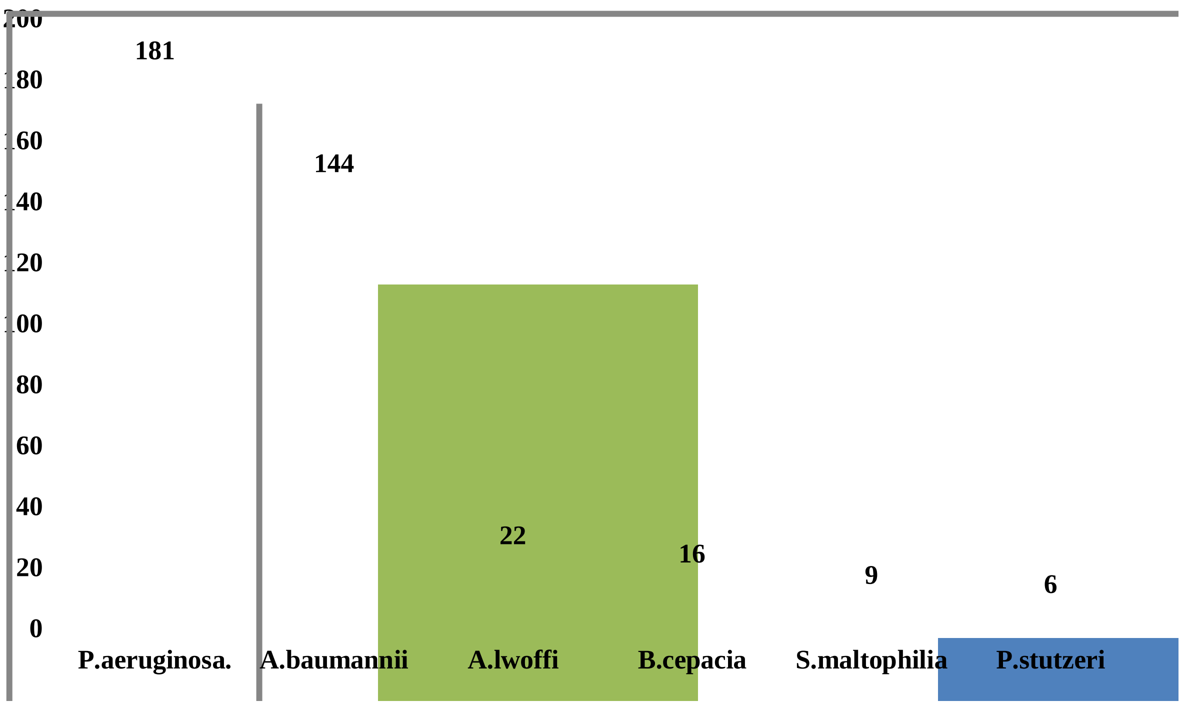
In present study, isolation rate of NFGNB from 3363 culture positive clinical specimen was 11.24% which is in parallel to other studies done by Grewal US *et al.,* Mahapatra A *et al.,* and Benachinmardi *et al.,*[15,16,17] Higher positivity rate of non-fermenters was reported by Rao *et al.,* (66.88%), and Dipak Bhargava *et al.,* (29.62%).[18,19]

Fig.1 Distribution of clinical specimens based on culture positivity



Numbers shown in figure are percentage.

Fig.2 Distribution of Non-fermenting gram-negative bacilli



Number shown in figure are absolute numbers.

Table.1 Sample wise distribution of NFGNB isolates

Specimen /Isolates	*P.aeruginosa.*	*A.baumannii*	*A.lwoffi*	*B.cepacia*	*S.maltophilia*	*P.stutzeri*
Pus (143)	61 (33.70)	76 (52.78)	00 (0.0)	00 (0.0)	03 (33.33)	03 (50.0)
Urine (64)	45 (24.86)	08 (05.56)	11 (50.0)	00 (0.0)	00 (0.0)	00 (0.0)
Sputum (33)	09 (04.97)	07 (04.86)	05 (22.73)	12 (75.0)	00 (0.0)	00 (0.0)
Wound swab (62)	41 (22.65)	16 (11.11)	03 (13.64)	00 (0.0)	02 (22.22)	00 (0.0)
Blood (31)	11 (06.08)	15 (10.42)	02 (09.09)	00 (0.0)	01 (11.11)	02 (33.33)
Body fluid (26)	09 (04.97)	16 (11.11)	00 (0.0)	00 (0.0)	01 (11.11)	00 (0.0)
Others (19)	05 (02.76)	06 (04.17)	01 (04.55)	04 (25.0)	02 (22.2)	01 (16.67)
Total (378)	181	144	22	16	09	06

Table.2 Distribution of non-fermenters based on antimicrobial sensitivity pattern

Specimen /Antimicrobials	*P.aeruginosa.* (n=181)	*A.baumannii* (n=144)	*A.lwoffi* (n=22)	*B.cepacia* (n=16)	*S.maltophilia* (n=9)	*P.stutzeri* (n=6)
Ciprofloxacin	86 (47.51)	73 (50.69)	12 (54.55)	06 (37.50)	09 (100)	04 (66.67)
Gentamicin	83 (45.86)	75 (52.08)	11 (50.0)	-	-	03 (50.0)
Amikacin	124 (68.51)	111 (77.08)	18 (81.82)	-	05 (55.56)	04 (66.67)
Ceftazidime	119 (65.75)	92 (63.89)	19 (86.36)	-	-	04 (66.67)
Ceftriaxone	123 (67.96)	93 (64.58)	19 (86.36)	-	-	-
Cotrimoxazole	-	75 (52.08)	12 (54.55)	16 (100)	09 (100)	-
Imipenem	153 (84.53)	113 (78.47)	22 (100)	12 (75.0)	-	06 (100)
Meropenem	154 (85.08)	116 (80.56)	22 (100)	11 (68.75)	-	06 (100)
Piperacillin/Tazobactum	149 (82.32)	111 (77.08)	22 (100)	07 (43.75)	04 (44.44)	06 (100)
Polymyxin-B	181 (100)	144 (100)	22 (100)	-	09 (100)	06 (100)

In the current study, predominantly isolated NFGNB was Pseudomonas aeruginosa followed by *Acinetobacter baumanii*, which comply with the results of Jayanthi *et al.,* and Bhargava *et al.,* however Goel *et al.,* and Samanta *et al.,* found *A. baumannii* as common species isolated. [20,21,22,23]

In present study, NFGNB were most frequently isolated from pus (37.83%) samples followed by urine samples (16.93%) while Mandira Sarkar *et al.,* isolated NFGNB most frequently from urine (29.44%) and pus (27.49%).[14]

In present study most common isolate, *P. aeruginosa* were found to be most susceptible to Polymyxin B (100%), which is similar to the findings of study done by Udhayvir Singh Grewal *et al.,*[15] Although Polymyxin B is not routinely used to treat infections caused by *P. aeruginosa* and is only tried as a last resort in case of severe multidrug‑ resistant Gram‑ negative bacterial infections. Nearly 84.53% and 85.08% of the *P. aeruginosa* isolates were found to be sensitive to Imipenem and Meropenem. Similarly, Malini *et al.,* and Rit *et al.,* documented over 90% susceptibility to Imipenem and Meropenem. [24,5]

The prevalence, species and sensitivity of NFs often varies in-between and within communities and hospitals. Faced these situation, the physician in clinical practice must be updated with prevalence and antimicrobial susceptibility pattern of these NFs and has the responsibility of making clinical judgments for use of appropriate antibiotics.

Observation from present study showed that the NFGNB which was thought to be commensals or contaminants are now emerging as important nosocomial pathogens. Our study showed a significantly high prevalence (11.24%) of NFGNB, the most common being *P. aeruginosa* and A. baumannii. Most of the common NFGNB showed resistant for commonly prescribed antibiotics although sensitive to higher group of antibiotics. Isolation of NFGNB and their antibiotic susceptibility pattern should be regarded with all gravity in clinical epidemiology because by being resistant to multiple antibiotics, their prevalence not only limits the treatment options but also act as a reservoir of drug resistance genes.

Recommendations

A multicentric cohort study including various clinical settings and professionals must be conducted at large scale with strictly following the guidelines. All the health care institutions must developed institutional antibiotic policy by considering sensitivity pattern of NFs in their community and in light of standard protocol. All professionals must be updated regarding change in susceptibility pattern and drugs of choice for management of concern infection.

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How to cite this article:

Rajesh Bansal, Rahul Soni and Yogendra Kumar Tiwari. 2019. Prevalence of Non - Fermenters among Various Clinical Samples and Their Antibiotic Resistance at Tertiary Care Centre Jhalawar. *Int.J.Curr.Microbiol.App.Sci.* 8(07): 1851-1858.
doi: https://doi.org/10.20546/ijcmas.2019.807.220