Isolation, identification and antibiogram of Oxidase negative non fermenting gram negative bacilli from the clinical specimens

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Abstract

Introduction: Oxidase negative nonfermenters are increasingly being isolated from the clinical specimens. Organisms like acinetobacter have been associated with multidrug resistance which makes the treatment difficult. Proper identification and determination of the antibiogram will help in appropriate management of the patients.

Aims and Objectives: The study aims to isolate the oxidase negative gram negative bacilli from the clinical specimens and identify them up to the species level and to know the antibiotic susceptibility pattern of these isolates.

Materials and Methods: All the clinical specimens obtained in the microbiology department were processed according to the standard protocols. Organisms were isolated and identified by using conventional biochemical testing methods. Antibiotic susceptibility was done by Kirby Bauer disc diffusion testing following the CLSI standards.

Results: A total of 153 oxidase negative NFGNB were isolated from the clinical specimens. Out of these Acinetobacter species constituted 146(95%) of the total isolates and the remaining 7(4.5%) were identified as Stenotrophomonas maltophila. The most common species of Acinetobacter isolated was Acinetobacter baumannii complex (Acb- complex), accounting 107(70%) of the total isolates followed by A.lowffii 25(16%), A. junii 10 (7%) and A.hemolyticus 4(2.7%). Majority of the Acb-complex were isolated from respiratory secretions (50.4%) followed by pus samples (30.8%). Stenotrophomonas species were also isolated predominantly from respiratory secretions (71.4%). The most effective antibiotic against these NFGNB were polymyxin B (98% sensitive) followed by tigecycline (96% sensitive). Cotrimoxazole retained its susceptibility against Stenotrophomonas maltophila with all the isolates being susceptible.

Conclusion: Speciation of oxidase negative NFGNB has gained importance because of the diverse species involved and their varied antibiogram patterns. Identifying the etiological agents and their susceptibility patterns will help in the better management of patients and reduces mortality and morbidity associated with these infections.

Keywords: Non fermenters, Acinetobacter, Drug resistance.

Introduction

Non fermenting gram negative bacilli are a group of aerobic, non sporing organisms that either do not utilize carbohydrates as a source of energy or degrade them through metabolic pathways other than fermentation. Previously the significance of these organisms was underestimated because of relative infrequent recovery of these organisms and slow growth and also lack of familiarity in identification. But now these organisms are being isolated more commonly from the clinical specimen implicating their role in pathogenesis.

They occur as saprophytes distributed widely in soil and water[1] and have emerged as important healthcare associated pathogens. Non fermenting gram negative bacteria are known to account for about 15% of all bacterial isolates from a Clinical Microbiological laboratory.[2] Though oxidase positive organisms like Pseudomonas is well characterized and easy to identify, the identification of oxidase negative non fermenting gram negative bacilli like *Acinetobacter* and *Stenotrophomonas maltophilia* is always cumbersome and speciation of these organisms is even more difficult.[3,4]

These organisms are known to cause many hospital acquired infections and the infections caused by these organisms are on rise. Acinetobacter and Stenotrophomonas maltophilia have been increasingly isolated from infections of respiratory tract including pneumonia (most often related to endotracheal tubes or tracheostomies), endocarditis and meningitis. Skin and wound infection, peritonitis (in patients receiving peritoneal dialysis) and urinary tract infections. Sporadic cases of conjunctivitis, osteomyelitis and synovitis have also been reported. It is now recognized that *Acinetobacter* and *Stenotrophomonas maltophilia* play a significant role in the colonization and infection of hospitalized patients.[5]

Most Acinetobacter are drug resistant. Therapy for carbapenam-resistant Acinetobacter is particularly problematic. Similarly, majority of strains of *S. maltophilia* are characterized by their resistance to many currently available broad spectrum antimicrobial agents. Most reports in India do not report the species involved in human infections and address the infections only at genus level. Speciation of isolates is important in the epidemiology of these infections. It is therefore necessary to speciate these organisms from various clinical samples and determine their sensitivity pattern.

Aims and Objectives

To isolate and identify the non-fermenting gram negative bacilli up to species level and to study the antibiogram of these organisms.

Materials and Methods

This prospective study was conducted in the Dept. of Microbiology, S.V.S Medical College, Mahabubnagar.

Inclusion Criteria

Only oxidase negative non fermenting gram negative bacilli (NFGNB) were included in this study.

Exclusive Criteria

All oxidase positive gram negative organisms were excluded from the study.

Methodology

Various clinical samples such as pus, blood, sputum, urine, CSF etc., were examined for isolation and identification of NFGNB and antimicrobial susceptibility testing. Presumptive identification of non-fermenters was made by inoculation on MacConkey Agar medium and incubated at 37^0C for 24 hrs. Non-lactose fermenters isolated, were subjected for oxidase test. All oxidase negative organisms were isolated and identified by a battery of tests like motility, study of cultural characteristics on Blood Agar, MacConkey Agar and biochemical tests like Catalase Oxidase, Urease, Arginine dihydrolase, Nitrate reduction, Citrate test, Malonate test Gelatin liquefaction test, Indole production test, Utilization of carbohydrates, Inoculation on triple sugar iron Agar, Oxidation fermentation test (Hugh Leifson method) Esculin hydrolysis and Growth at 42^0C. Based on these phenotypic tests acinetobacter has been speciated into *A.bauamannii complex*, *A.lwoffi, A.junii* and *A.hemolyticus.*[1,5]

Antibiotic Susceptibility testing was done by by Kirby Bauer Method

Commercially obtained Hi media discs were used. The strength of the disc and their zone size used were according to the guidelines by CLSI guideline standards.[6]

For tigecycline and polymyxin B there are no CLSI disc diffusion guidelines (except for polymyxin B against pseudomonas). In the present study for tigecycline disc diffusion breakpoints adopted by Jones et al[7] was used. For polymyxin B testing, the zone size of pseudomonas (CLSI) was adopted for all the NFGNB. Those with less than 10mm zone size were further tested for MIC by E-strips (Hi-Media). *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

Statistical Analysis

Data entry, data base management and analysis was done with the use of SPSS software (Version 16). Descriptive statistics was used to calculate the frequency, mean and percentage. The frequency of distribution of organisms in accordance with the sample and their resistance patterns has been analyzed and depicted in the form of tables, bar diagrams and/or pie diagrams.

Results

A total of 1632 species of Gram negative bacilli were isolated from various samples obtained from patients admitted in various wards like surgical, medical and ICU in S.V.S. Hospital Mahabubnagar, Telangana. Among 1632 GNB, nonfermenting, oxidase negative organisms accounted for a total of 153(9.3%).

When these nonfermenters are identified to the species level the predominant organism isolated was *Acinetobacter* constituting 146(95%) of the total isolates and the remaining 7(4.5%) identified as *Stenotrophomonas*. In the present study, the organisms were predominantly isolated from males (67%) than females (33%)

Table 1: Distribution of non-fermenters

Species	No. of isolates	Percentage
Acinetobacter	146	95.43%
S.maltophilia	07	4.57%

The most common species of Acinetobacter isolated was *Acinetobacter baumannii* complex (Acb- complex), constituting 107(70%) of the total isolates followed by *A. lowffii* 25(16%), *A.junii* 10 (7%) and *A.hemolyticus* 4(2.7%). *Stenotrophomonas maltophila* has contributes to an account of 7(4.5%).

Table 2: Species wise distribution of isolates

S. No	Species	No. of Isolates	Percentage
1	*Acb - complex*	107	70%
2	*A.lwoffi*	25	16%
3	*A.junii*	10	7%
4	*A.hemolyticus*	4	2.5%
5	*S.maltophilia*	7	4.5%
	Total	153	100%

Majority of the Acb-complex were isolated from respiratory secretions accounting to 54(50.4%) out of 107 isolates followed by pus samples accounting to 33(30.8%). The second most common species A.lwoffi was also isolated predominantly from respiratory secretions accounting to 12(48%) out of 25 isolated. *A.junii* were predominantly isolated from pus samples accounting 6(60%) out of 10 and *A.hemolyticus* from blood samples 2 out of 4 isolates. Majority of the *Stenotrophomonas* species were also isolated from respiratory secretions 5 (71.4%) out of 7 isolates.

145(95%) out of 153 NFGNB isolates were obtained as the hospital based isolates and only 8(5%) were from the out patients. Out of these 145 in-patient isolates, 87(60%) were from the intensive care units and acute medical care units and the remaining 58(40%) were isolated from the wards including surgical wards 26% followed by medical wards 14%.

The most effective antibiotic against NFGNB was polymyxin B with only 2% of the isolates showing resistance and the remaining 98% being sensitive to it. Polymyxin B was followed by tigecycline with respect to susceptibility showing only 4% of the total isolates resistant to it and maintaining its efficacy against majority these pathogens. Along side of tigecycline, imipenem also showed considerable sensitivity against these pathogens with 27% of isolates showing resistant to it and the remaining 73% being sensitive. The highest resistance was observed against ceftazidime with 79% of the isolates showing resistance to this antibiotic. This is followed by cefipime with 65% isolates showing resistance and ciprofloxacin with 61% isolates being resistant. Resistance to other important antibiotics like piperacillin tazobactum

(56%), cefaperazone sulbactum (43%), amikacin (41%) was also notably high.

Table 3: Sample wise distribution of NFGNB

Specimen	*Acb - complex*	*A.lwoffi*	*A.junii*	*A.hemolyticus*	*S.maltophilia*	Total
Urine	8	1	0	0	0	9
Blood	3	1	0	2	0	6
Resp secret	54	12	4	1	5	76
CSF	2	2	0	0	0	4
Pus/Swabs	33	7	6	1	2	49
Body fluids	7	2	0	0	0	9
Total	107	25	10	4	7	153

Table 4: Antibiotic senstivity pattern

Total-153	Sensitive %	Resistant%
Amikacin	53	47
Cefipime	35	65
Ceftazidime	21	79
Imipenam	73	27
Piperacillin/ Tazobactam	44	56
Polymyxin B	98	2
Doxycycline	59	41
Ciprofloxaclin	39	61
Co-trimoxazole	47	53
Tigecycline	96	4
Cefoperazone/Sulbactam	57	43

Table 5: Percentage of resistance pattern (species wise)

S. No	Species	Total	AK	CPM	CAZ	IPM	PIT	PB	DO	CIP	COT	TGC	CFS
1	Acb - complex	107	58%	78%	84%	30%	60%	2%	46%	71%	64%	4%	50%
2	A.lwoffi	25	4%	20%	44%	0	28%	0	8%	20%	16%	0%	0
3	A.junni	10	30%	70%	90%	30%	80%	0	60%	70%	50%	0%	60%
4	A radio	4	75%	100%	100%	0	75%	0	75%	75%	75%	0%	75%
5	S.maltophilia	7	43%		100%	100%	43%		29%	29%	0	0	43%

Regarding the species, A.hemolyticus had shown highest resistance to third generation cephalosporins, with all the isolates (100%) showing resistance to ceftazidime. A.junii and Acb-complex have also showed very high resistance to ceftazidime with 90% and 84% of the isolates show in resistance to it respectively. Least resistance was seen in A.lwoffi with only 44% isolates being resistance to it.

The highest resistance to imipenem was observed among Acb-complex and A.junii with 30% of each showing resistance to it. Polymyxin B resistance was observed only among Acb-complex with 2% of the isolates being resistant. Other isolates have not shown resistance to this antibiotic. Tigecycline resistance was also seen only among Acb-complex (4%).

In the present study A.lwoffi had shown the least resistance to any antibiotic when compared to the other species.

All the isolates of Stenotrophomonas were uniformly sensitive to co- trimoxazole and tigecycline showing 100% sensitivity. Ciprofloxacin and doxycycline have also shown considerable sensitivity with only 29% of isolates being resistant to each of them.

Overall, polymyxin B and tigecycline have been the highly sensitive drugs against any of the above non fermenting gram negative bacilli and imipenem being the next.

Discussion

Nonfermenting gram-negative bacilli (NFGNB), are being increasingly implicated in human disease and have emerged as important healthcare-associated pathogens. The complex physiochemical properties of these organisms necessitate a battery of tests for their precise identification. Identification of these nonfermenters has often being neglected. They exhibit resistance not only to beta lactam and the other groups of antibiotics, but also to carbapenems.

In the present study we intended to identify commonly encountered, clinically significant gram negative nonfermenting bacteria from clinical specimen along with their antimicrobial susceptibility pattern.

In our study out of 1632 gram negative isolates, 153 were oxidase negative non fermenting gram negative bacteria, with a prevalence rate of 9.3% among the total isolates. This finding is similar to Joshi et al[8] study where the prevalence rate was 9.6%. Other studies like Dash et al,[9] Mostofi et al[8] showed prevalence rates between 4.5% to 14% respectively.

In our study most of the isolates of NFGNB were from respiratory samples, similar to the observation made by others like Sinha et al,[10] Seifert et al.[11] Non fermentive gram negative bacilli especially Acinetobacter is known to be predominantly associated with respiratory infections especially in debilitated patients who are on ventilator. Ventilator associated pneumonia is one such a complicated nosocomial infection which is difficult to treat and associated with high mortality and morbidity

Bacteremias associated with NFGNB are not uncommon. They may occur either monomicrobial or as polymicrobial infections. In our study blood isolates constituted to an account of 6(3.9%) out of 153 isolates. Though this percentage is considerably less, the mortality associated with these infections is significantly high as most of these infections are highly drug resistant. In Dash et al[9] study 13.1% of the total isolates were from the blood samples. All these isolates were isolated as nosocomial pathogens predominantly from the intensive care units. No community acquired bacteremias were reported. These findings are similar to our study where all the blood isolates were from the nosocomial set up and all were isolated from the neonatal and paediatric intensive care units.

In the present study majority of the isolates were isolated as nosocomial pathogens and only a minority were obtained from the out patients. 145(95%) out of 153 isolates were obtained as the hospital based isolates and only 8(5%) isolates were from the out patients indicating community acquired infections. These findings are similar to Dash et al[9] and Jaggi et al[12] studies.

Among Acinetobacter isolates Acb complex was the most commonly isolated species observed in our study constituting 70% of the total Acinetobacter isolates. These findings are similar to the studies conducted by Seifert et al,[11] Sinha et al,[10] Joshi et al,[8] Dash et al[9] where Acinetobacter baumanni was the most common isolated species constituting 72.9%, 75%, 70%, 79.6% respectively.

The second most commonly isolated species in our study was A.lwoffii constituting 17% of the total Acinetobacter isolates. Majority of the A.lwoffii was isolated from the respiratory specimens (48%), pus samples (28%). Only 1 isolate (4%) was from the blood samples. Similar observations were made by Sinha et al[10] and Dash et al[9] where the A.lwoffi was the second common isolate constituting about 24% and 12% of the total Acinetobacer isolates respectively. In contrast to our study Joshi et al[8] study has isolated A.junii as the second most common species isolated accounting to 8.6% of the isolates.

The least common isolate in our study was A.hemolyticus accounting to only 2.6% of the total Acinetobacter. Half of the isolates (50%) were isolated from the blood samples followed by pus and respiratory samples (25% each). This finding was almost similar to many other studies conducted by different researchers where majority of them have not isolated this species like Seifert et al.[11] In Joshi et al[8] study, A.hemolyticus accounted for 6.4% of the total Acinetobacter isolated but it was predominantly isolated from the wound exudates (89%) and none from respiratory and blood specimens.

Susceptibilities of NFGNB to different antimicrobials varies from species to species and also from place to place among the same species. The resistance rates of Acinetobacter isolated from different parts of the world varies widely. Because of this local surveillance studies are always important to know the most adequate therapy for treating these infections

In our study majority of the Acb complex were resistant to third generation cephalosporin, Ceftazidime with 84% of the isolates being resistant to it. Similar findings were observed in studies conducted by Dipendra et al,[15] S. Mohanty et al,[14] and Sinha et al[10] where ceftazidime resistance was 74%, 84% and 74% respectively.

This was followed by high resistance to cefepime (79%), ciprofloxacin (71%) and piperacillin tazobactum (60%). Almost similar rates of resistance was observed in the other studies by Sinha et al[83], Dipendra et al,[15] Dash et al[9], where the cefepime resistance ranged between 74% to 89%, Ciprofloxacin resistance ranged between 64% to 86%, piperacillin tazobactum resistance ranged between 23% to 90%.

Carbapenems are considered to be the drug of choice for the treatment of Acinetobacter infections. In India, carbapenems are widely used as a last resort in infections due to multidrug resistance Acinetobacter. In recent years there have been reports of reduced susceptibility to these drugs from various parts of the country. In our study, the resistance of Acb complex to imipenem was 30%. There is a considerable variation in the susceptibility rates of imipenem from study to study. In Sinha et al[10] study the resistance rate was 14% and in S. Mohanty et al[14] study this was as high as 64%. Other studies where the resistance rate is similar to our study include studies by Taneja et al 25% resistance[9] and Dash et al 19% resistance.[9] This high resistance to carbapenems in our study is of great concern as there will be very few options left for the treatment of these infections.

Table 6: Comparative species distribution in different studies

Study	A.baumannii	A.lwoffi	A.junii	A.hemolyticus	Other spp	Total
Dash et al[9]	79.6%	12.4%	8%	-	-	100%
K.Prashanth et al[13]	71.1%	20.3%	1.6%	3.38%	3.6%	100%
Joshi et al[8]	70%	-	8.6%	6.4%	15%	100%
Sinha et al[10]	75%	24%	1.3%	-	-	100%
Seifert et al[11]	73%	3.6%	1.9%	-	21%	100%
Our Study	73%	17%	6.8%	2.7%	-	100%

The most effective drug against Acb complex in our study were polymyxin B (colistin) with only 2% of the isolates being resistant to it. Polymyxins are relatively older drugs used very early in the antibiotic era. But because of the nephrotoxic potential these drugs have been under dormant stage for all these years masked by the effective groups of drugs like carbapenems which are considered safer drugs. But with the emergence of resistance to carbapenems, polymyxins have once again gained their importance as potential therapeutic agents against carbapenem resistant Acinetobacter. The relatively low percentage of resistance to polymyxins in our study is a fruitful result, retaining its potential for treatment, comparable with the studies conducted by Taneja et al (3.5%)[16] and Dash et al[9] (0%) resistance. Other studies with similar rate to our study was the study by S. Mohanty et al (6%) resistance.[14]

The other newer and effective drug against these pathogens is tigecycline. Various authors have reported the resistance rates to tigecycline to vary from being nonexistent to 66%.[17,18] Studies have documented that overexpression of multidrug efflux pump regulated by adeABC efflux pump has a major role in the resistance towards tigecyline.

The second NFGNB isolated in our study apart from Acinetobacter was Stenotrophomonas maltophilia. The isolation rate of this organism among total isolates was 4.5% (7/153). This is similar to the study by Nautyal et al[19] where the isolation rate from the total NFGNB (both oxidase positive and negative) was 1.8%, and when considered only oxidase negative organisms this rate was 6.1%.

Majority of the Stenotrophomonas were isolated predominantly from respiratory samples accounting to 71.4% of the total Stenotrophomonas isolates. This is similar to Chung et al [20] study where 51% of the isolates were from respiratory samples.

In our study Stenotrophomonas has shown highest resistance to ceftazidime (100%), imipenem (100%), followed piperacillin tazobactum (43%), cefaperazone sulbactum (43%). These findings were nearer to the findings of Chung et al[20] Otkun et al,[21] Nautyal et al.[19] where the resistance to ceftazidime ranged from 32% to 69%, piperacillin tazobactum resistance ranged from 39% to 75%, cefaperazone sulbactum resistance around 38%. Fluoroquinolones resistance in our study was 29% which was similar to Chung et al study (30%).[20]

In the present study cotrimoxazole has shown absolute sensitivity (100% sensitive) against all the isolates of Stenotrophomonas. This correlates well with the findings of Chung et al (94%) sensitive,[20] Otkun et al (98%) sensitive.[21] Tigecycline has also shown absolute sensitivity against this organism which is similar to Chung et al[20] where all the isolates were sensitive to it.

Despite many intensive efforts, the nosocomial acquisition of Acinetobacter remains problematic, especially in intensive care units. There are difficulties both in control and nosocomial infection due to their high resistance to antimicrobials in hospital environment. Susceptibilities to Acinetobacter spp. against antimicrobials is considerably different among countries, centers and even among the wards of a given hospitals therefore such type of local surveillance studies are very important in deciding the most adequate therapy for Acinetobacter infections. The development of resistance to antimicrobials in Acinetobacter appears to be unstoppable and MDR isolates of Acinetobacter are increasing day by day and this is one of the reasons for the rapid spread of resistance. Resistance to carbapenems is also increasingly observed now a days. Polymyxin B and colistin have demonstrated reasonable success in the control of these drug resistant isolates but these have their own disadvantages of being nephrotoxic drugs and also monotherapy with these drugs may lead to selection of the heteroresistance populations for which the combination therapy is the only option available for treatment.

Conclusion

Though identification of the non-fermenting gram negative up to the species level is a difficult task, with the gaining importance of particular organisms like Acb complex and stenotrophomonas and with their unique resistance patterns it has become important to identify and know the antimicrobial susceptibility patterns which in turn will help in not only treating the patients but also in the infection control practices.

Conflict of Interest: None.

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