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Prevalence of Nonfermenting - Gram Negative Bacilli from Urinary Tract Infection

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***Abstract:** Aerobic nonfermenting gram-negative bacilli (NFGNB) are now emerging as importantUropathogens. Methods: This study was done to know the significance of NFGNB other than P.aeruginosa and Acinetobacter spp. in Urinary Tract Infections (UTI). Total 660 urine specimens received in three months period from March 2019 to May 2019 were subjected to semi-quantitative culture as per the standard procedures in the routine microbiology laboratory and the results were noted. Detailed clinical history and laboratory parameters (Total count, Urine microscopy: Pus cell and RBC) were gathered to know the significance of the organism. Results: Total 4. 09% of the NFGNB isolates were clinical significant. Common risk factors associated with these NFGNB are ICU stay, previous hospitalization, catheterization and Diabetes Mellitus. Conclusion: Clinical correlation of NFGNB from urine is required before considering them clinically significant or contaminants.*
***Keywords:** Urinary tract infection; Nonfermenting gramnegative bacilli; Risk factors*

I. INTRODUCTION

Urinary tract infection is the second most common bacterial infections affecting humans throughout their lifetime. Escherichia coli is the commonest Urinary tract pathogen accounting for over 80% of community acquired infection. The nonfermentative gram-negative bacilli are a heterogenous group of aerobic, non-spore forming bacilli that either do not use carbohydrates as a source of energy for degrade them through metabolic pathways other than fermentation.these nonfermenters include organisms from diverse genera like Pseudomonas, Acinetobacter, Alcaligenes, Myroides, Oligella, Flavimonas, Agrobacter Weeksiella, etc. Pseudumonas aeruginosa is the predominant and most well-known organism out of this heterogeneous group. This is partly due to its easy recognition in the laboratory as it produces pyocyanin=a blue- green pigment , Pyorubin-red pigment , pyoverdin =yellow-green pigment, pyomelanin=brown -black pigment.They occur as saprophytes in the environment and some are also found as commensals in the human gut.[5] NFGNB also known as Non-fermenters (NFs) are emerging with increasing frequency as agents of opportunistic and often, serious infection as well as nosocomial infection.[2,1].

They are frequently isolated from cases such as septicemia, meningitis, pneumonia, urinary tract infections& surgical wound infection. [4,1] Among the species that are opportunistic pathogens in immunologically compromised host either by disease or treatment, Pseudomonas aeruginosa (P. aeruginosa) is eminent, followed by Acinetobacter baumanii (A. baumanii), P.fluorescence, P.stutzeri, Stenotrophomonas maltophilia, P.putida, P.cepacia[5]. According to previous studies done Isolation rates for NFGNB in Urine samples was 10.8-18% with the majority organisms are Pseudomonas aeruginosa, followed by Acinetobacter spp.

II. MATERIAL AND METHOD

A. Materials & Methods

This study comprises of 660 samples of clinical specimens, which was collected from IPD and OPD patients from the various departments of Surat Municipal Institute of Medical Education & Research (SMIMER), Surat during a period of 3 month from March 2019 to May 2019 .

B. Urine

Smears for Gram's stain were made by placing a loopful urine sample on a clean slide and allowed to air dry. These samples were then plated by a 4mm loop onto 5 % sheep BA and MA for semi-quantitative analysis also inoculate on Macconkey agar for differentiation to aid to identification. Plates will be incubate for 24 hrs at 37 degree C

Only those isolates, which found significant in semi quantitative culture of urine, were included in the study.

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III.NON-FERMENTATIVE GRAM-NEGATIVE BACILLI (NFGNB) WILL IDENTIFY UP TO GENUS OR SPECIES LEVEL BASED ON THE FOLLOWING TEST:

1.Grams stain. 2.Oxidase test. 3.Motility test. 4.pigment production. 5.Indole test. 6.Urease test. 7.Citrate test. 8.Utilization of 10% lactose. 9.Decarboxylation of lysine & Ornithine. 10.Catalase test. 11.Oxidative fermentation(OF) of (Hugh-Leifson) Glucose(G), lactose(l), Mannitol(M), Xylose(X). 12.Gelatin liquefaction. 13.Esculin hydrolysis

A. Oxidase Test

1) *Filter Paper Strip Procedure:* Take a filter paper strip. Moisten the filter paper strip with freshly prepared 1% OXidase reagent. Pick up the colonies to be tested with the help of a glass rod or plastic loop or platinum wire. Smear the colonies into the reagent zone of the filter paper. Note the change color if any within 10 sec. In a positive test, a deep blue color develops at the site of smear in the filter paper. within 10 sec. In a negative test the color of the smear in the zone of the filter paper remain unchanged.

B. Catalase Test

1) *Procedure:* Test can be done by 2 methods, namely Slide method and Tube method.
2) *Slide Method:* Transfer pure growth of the organism from the nutrient agar to a clean slide with a loop or glass rod. Immediately add a drop of 3% hydrogen peroxide to the growth. Observe for bubble formation. Tube method: Take 1 ml of 3% hydrogen peroxide in test tube.Introduce small quantity of bacterial growth into the fluid with the help of a glass rod or plastic loop and touch the side of the tube. . Observe the release of bubbles.

C. Motility (Motility agar stab Techpnique)

1) *Procedure:* Sterilize the straight inoculating wire by flaming it in a Bunsen's flame. Dip the sterile wire into the culture of the test organism. Inoculate the culture into the medium by stabbing the medium right into the center of agar. Puncture the entire depth of the medium. Withdraw the wire back in the same straight line. Sterilize the wire and set it aside. Incubate the medium into incubator at 37□ for overnight. Next day observe the stab agar medium and look for the turbid growth across the line of inoculation, which indicates that the organisms are motile

D. Citrate Utilisation Test

1) *Procedure:* Using sterile technique, inoculate each bacteria into its appropriately labeled tube by means of a stab and streak inoculation. Incubate all culture for 24 hours to 48 hours at 37°C.Look for the development of deep blue color within 24-48 hours of incubation of the inoculated tube.

E. TSI (Triple Sugar Iron)

This media used for detection of carbohydrate fermentation. This media contain glucose (0.1 %) , lactose (1%) and sucrose (1%) are three carbohydrates, other contents are peptone, NaCl, sodium thiosulphate, pH of the medium is alkaline, phenolred and ferric ammonium citrate are indicators. Media is prepared in slant and butt portion in same tube. Slant portion, exposed throughout its surface to atmospheric oxygen, is aerobic and butt protected from air and is relatively anaerobic. It was inoculated with long, straight wire. Colony recovered from agar plate touched with end of inoculating needle, which was then stabbed into the deep,, extending to within 3 to 5 mm of its bottom then incubated at 35°C for 18-24 hours.
If no carbohydrate fermentation reaction was alkaline slant and alkaline butt, If only glucose was fermented slant was alkaline and butt was acidic, if glucose fermented along with H2S produced, reaction was alkaline in slant and acidic butt along with H2S.(1) If glucose and lactose and /or sucrose are fermented, reaction will be acidic in both butt and slant and if gas is produced it can be seen in form of bubbles or lif

F. OF medium (Hugh and Leifson)

Two tubes were required for the OF test of each sugar. Each tube was inoculated with the suspected NFGNB, using a straight needle stabbing the medium three to four time halfway to the bottom of the tube. One tube of each pair was covered with a 1-cm layer sterile mineral oil or melted paraffin, leaving the other tube open to the air. Incubate both the tubes at 35°C and examined daily for several days.

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G. Gelatin Hydrolysis Medium

Nutrient gelatin tubes were inoculated with fresh isolates and incubated at 37°C for 7 days. Every 2-3 days tubes were place in a refrigerator for 2 hrs and examined for of media. Liquefaction of the tube was considered as positive test.

H. Lysine Decarboxylase Test

A well isolated colony of the test organism recovered on primary isolation agar were inoculated on two test tubes of Moeller decarboxylase medium, one containing the lysine& the other to be used as control tube devoid of amino acid. Overlay both tubes with sterile mineral oil to cover about 1 cm of the surface and incubate at 35°C for 18 to 24 hours.

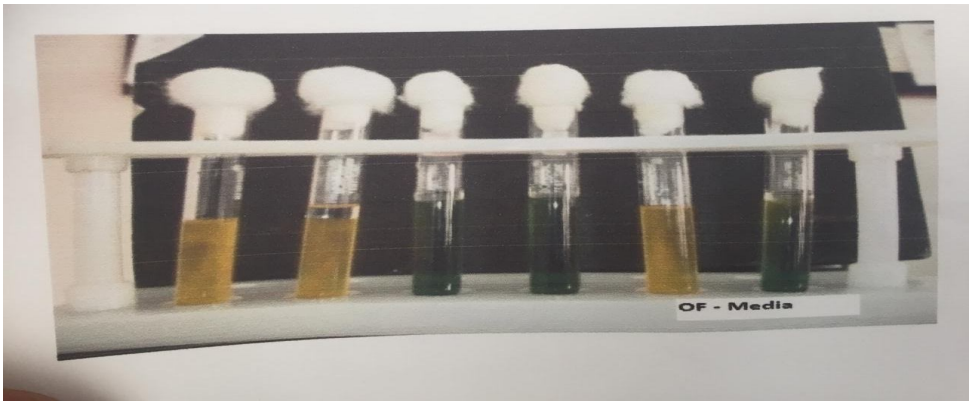
IV. RESULT AND DISCUSSION



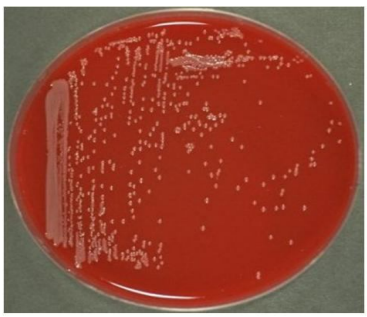
Pseudomonas aeruginosa on Gram staining



Pseudomonas on nutrient agar : Producing large, opaque, irregular colonies with bluish green pigmentation



OF reactions L-R fermentative, asaccharolytic&oxidative

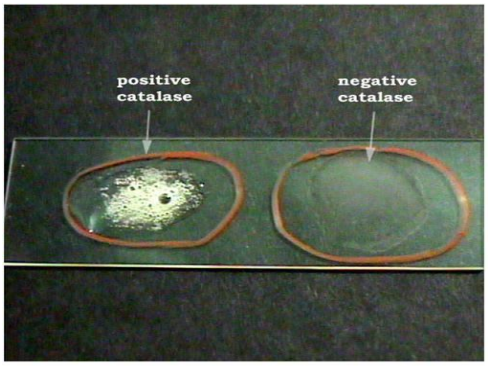


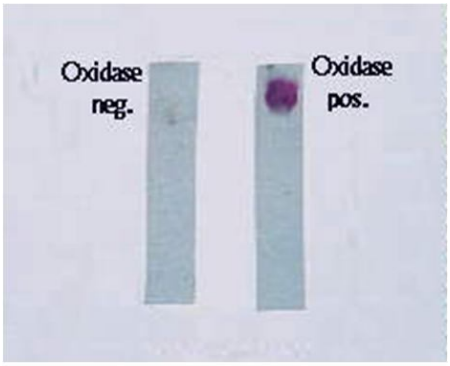
Pseudomonas fluorescence on Blood agar



Acinetobacter baumanii on Blood agar.

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Pseudomonas aeruginosa - catalase and oxidase test positive

Chart-1:Age wise distribution of clinical isolate of NFGNBs.

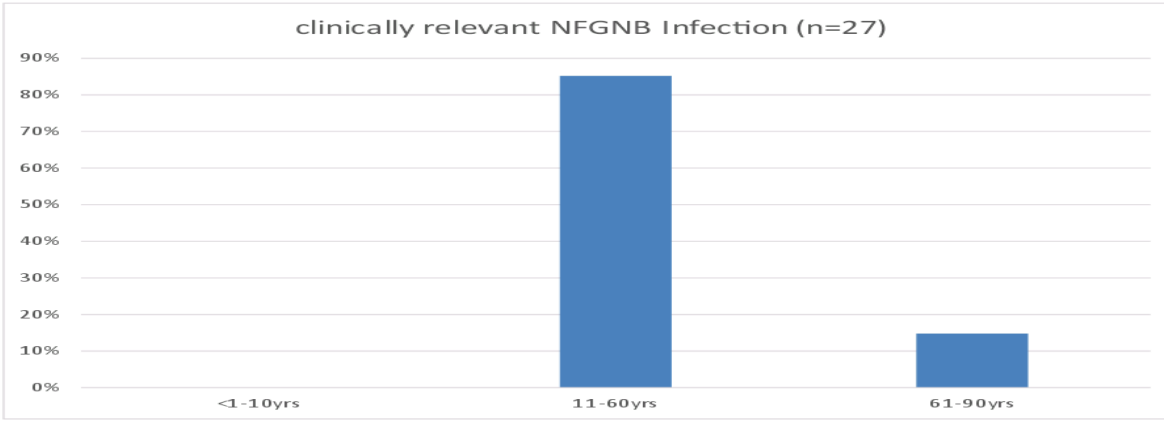
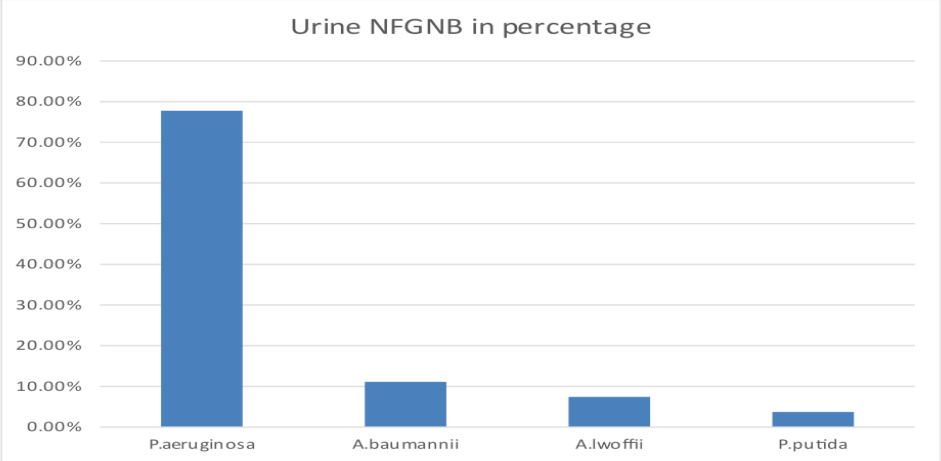


Chart 2: Various species of NFGNBs wise distribution

Organism	Urine NFGNB in percentage	NFGNB in number
P.aeruginosa	77.77%	21
A.baumannii	11.11%	3
A.lwoffii	7.40%	2
P.putida	3.70%	1

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V. CONCLUSIONS

The etiological role of NF isolated from differeat patients can be established by repeated Isolation of the same organisms.

A propective study was conducted to know the prevalence of non-fermenting gram negative bacilli isolated from urine specimen. Of the total bacterial isolates, 27 were Non-fermenting Gram negative bacilli recovered from urine samples. Of these. 21(77.77%) were Pseudomonas aeruginosa, 1(3.7%) were P.putida ,3(11.11%) were Acinetobacter baumannii and 2(7.4%) A .lwofiii.Most of the patients were having high risk factors like long stay ICUS, catheterization (both urinary and intravenous), diabetes, burns and malignancy. The mst common isolates were P.aeruginosa 77.77% followed by A.baumanii 11.11% , A.lwoffi 7.4%, and P.putida 3.7%. .

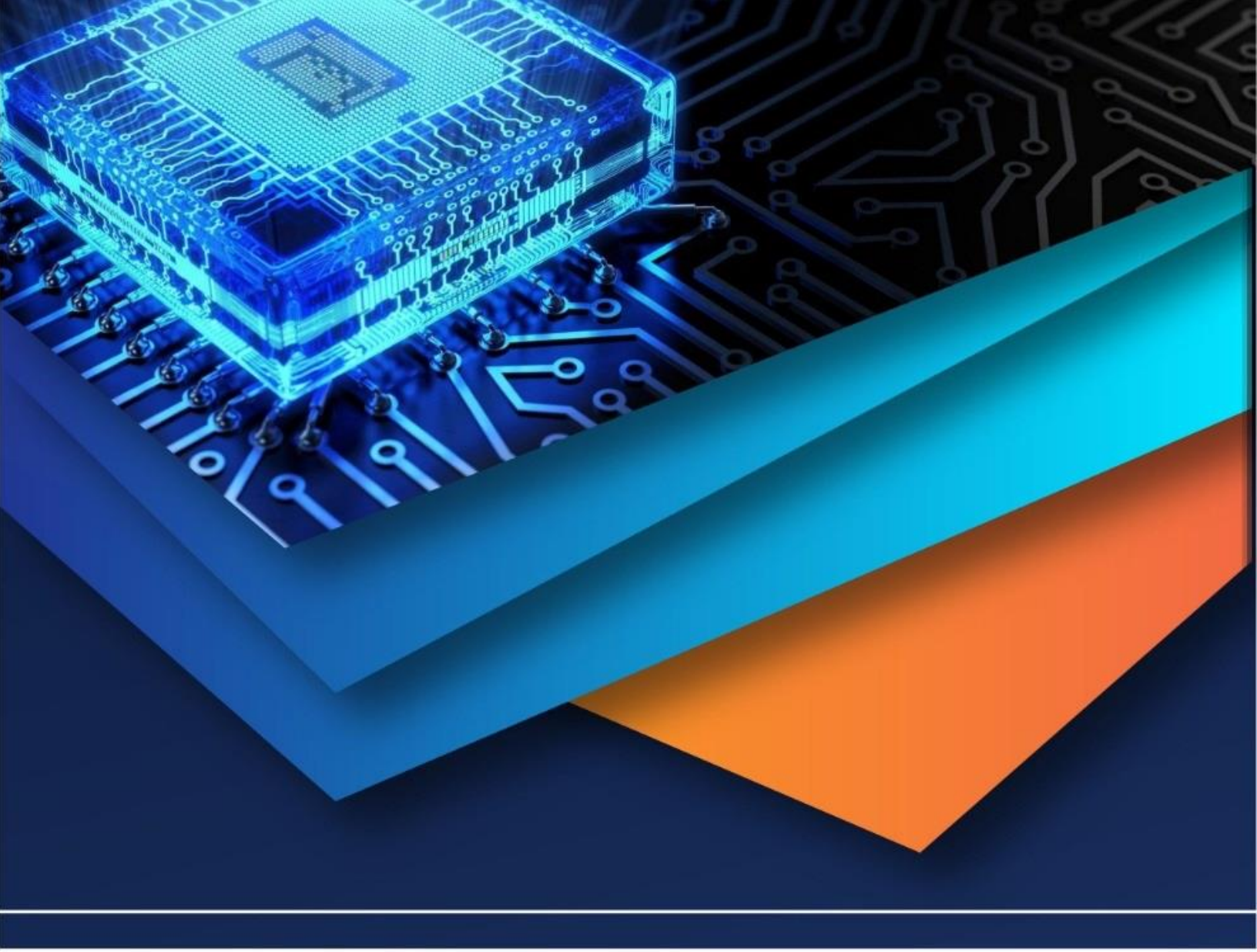
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