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Microbiology

CORRELATION OF DIRECT GRAM SMEAR FINDINGS WITH BACTERIAL ISOLATION IN SPUTUM OF PATIENTS.

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ABSTRACT

INTRODUCTION:-The Gram stain is a bacteriological laboratory technique used to differentiate between gram positive and gram negative bacteria. The most important primary test performed directly on clinical specimen is gram staining.

AIMS AND OBJECTIVE:- To correlate the gram stain findings with the bacteria isolated from the sputum samples obtained from patients

MATERIALS REQUIRED:-This is a retrospective study conducted for a period of 6 months (June 2018 and December 2018) at Department of Microbiology in Saveetha Medical College and Hospital, Chennai.

RESULTS:-The sputum collected from 109 patients from different departments was sent to the microbiology laboratory and following data has been arrived.

CONCLUSION:-1. Most common bacterial isolate from sputum was Pseudomonas aeruginosa.2. The presence of pus cells between 10 to 25 had a better correlation than that of pus cells less than 10.3. The epithelial cells had an inverse correlation with isolation of pathogen.

KEYWORDS : Gram Stain, Klebsiella Pneumonia, Sputum, Epithelial Cells, Pus Cells.

INTRODUCTION:-

The Gram stain is a bacteriological laboratory technique which is used to differentiate between gram positive and gram negative bacteria in the given sample. Gram positive and gram negative bacteria are differentiated based on the physical and chemical properties of the cell wall. Gram positive bacteria are composed of thick layer of peptidoglycan whereas gram negative bacteria are com- posed of thin layer of peptidoglycan.

Smears prepared directly from clinical specimens and stained by the Gram stain technique is a preliminary method for the identification of bacteria that is present in the sample, in addition to giving valuable information on the presence or not of pus cells which is an indicator of infection. Besides, the presence of numerous epithelial cells would prompt the laboratory technician to reject or still go ahead with the processing. When crystal violet is added, gram positive bacteria retains the violet color due to presence of thick peptidoglycan layer. At the same time gram negative bacteria can't retain the violet color when crystal violet is added since the peptidoglycan layer is thin.

The four basic steps of the gram staining technique include the following:

1) Apply crystal violet for one minute to a heat fixed culture smear. Heat fixation kills some bacteria but mostly affixes the bacteria to the slide to prevent rinsing during the process of staining.
2) Then add iodide for one minute so that iodide binds and traps the crystal violet within the cell.
3) Then add acetate for 30 sec for rapid decolorisation. Sometimes ethanol can also be used.
4) Finally add safranin for 30-60 sec for counterstaining. Carbon fuchsine may be used instead of safranin since it counterstains aerobic bacteria more efficiently.

The most important primary test performed directly on clinical specimen is gram staining. It is the most rapid and simplest test to characterize microorganisms. The information provided by gram staining will help to assess the adequacy of preliminary diagnosis and specific anti-microbial treatment.

AIM AND OBJECTIVES:-

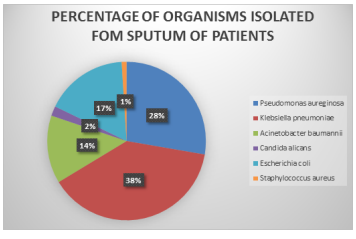
To correlate the gram stain findings with the bacteria isolated from the sputum samples obtained from patients

MATERIALS AND METHODS:

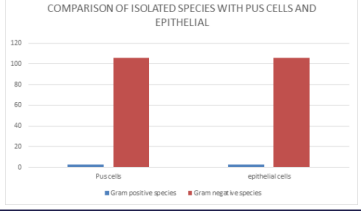
This is a retrospective study conducted for a period of 6 months (June 2018 and December 2018) at Department of Microbiology in Saveetha Medical College and Hospital, Chennai. A total of 109 sputum samples were collected from patients from general medicine, general surgery and OBG departments. Those samples were processed through gram stain and then inoculated onto chocolate agar, blood agar and Mac Conkey agar. Using carbon dioxide incubator those culture plates were incubated overnight at 37 degree Celsius. Next day gram stain was performed. Micro SCAN autoSCAN-4 instrument was used for finding antibiotic susceptibility pattern and for identification of organism isolated.

RESULTS:-

The sputum collected from 109 patients from different departments was sent to the microbiology laboratory and the different bacteria present in those sputum were isolated. From the bacteria isolated the following data has been arrived.



Isolated species		No.of Isolates	Percentage
Gram Negative organisms	Pseudomonas aeruginosa	30	28%
	Klebsiella pneumoniae	43	39%
	Acinetobacter baumnnii	15	14%
	Escherichia coli	18	17%
Gram Positive organisms	Candida albicans	2	2%
	Staphylococcus aureus	1	1%
Total		109	100%



CORRELATION OF PUS CELLS WITH ISOLATED BACTERIA

	<5	5-10	10-15	15-20	20-25	>25
P.aeruginosa	2	3	7	5	-	13
K.pneumoniae	4	3	9	7	8	11
A.baumanni	2	-	6	4	1	2
C.albicans	-	-	-	1	-	1
E.coli	2	1	4	4	-	5
S.aureus	-	-	-	-	-	1

CORRELATION OF EPITHELIAL CELLS WITH ISOLATED BACTERIA

	<5	5-10	10-15	15-20	20-25	>25
P.aeruginosa	12	7	4	7	1	-
K.pneumoniae	14	7	11	5	1	5
A.baumanii	-	5	4	3	-	2
C.albicans	1	-	1	-	-	-
E.coli	3	3	4	7	-	1
S.aureus	1	-	-	-	-	-

DISCUSSION:-

From the data collected,it is observed that the most common bacterial species isolated was klebsiella pneumoniae(39%).

Second most common organism isolated was pseudomonas auerginosa (28%) followed by Escherichia coli (17%) and, Acinetobacter baumanni (14%). Candida albicans (2%) and staphylococcus aerus (1%) are rarely found.<5 pus cells are predominantly seen in Klebsiella pneumoniae (4) followed by Pseudomonas aeruginosa (2), Acinetobacter baumanni (2) and Escherichia coli (2).

Candida albicans and Staphylococcus aureus do not contain pus cells in this category. 5-10 pus cells are found in Pseudomonas auerginosa (3), Klebsiella pneumoniae (3) and Escherichia coli (1).

Acinetobacter baumanni, Candida albicans and staphylococcus aureus do not contain pus cells in this category. 10-15 pus cells found in Klebsiella pneumoniae (9), Pseudomonas aeruginosa (7), Acinetobacter baumanni (6) and Escherichia coli (4). Candida albicans and Staphylococcus aureus don not contain pus cells in this category.
15-20 pus cells found in Klebsiella pneumoniae (7), Pseudomonas aeruginosa (5), Acinetobacter baumanni (4), Escherichia coli (4) and Candida al- bicans (1).

Staphylococcus aureus do not contain pus cells in this category. 20-25 pus cells are seen in Klebsiella pneumoniae (8) and Acinetobacter baumanni (1).

Pseudomonas aeruginosa, Candida albicans, Escherichia coli and Staphylococcus aureus do not contain pus cells in this category. >25 pus cells are seen in Pseudomonas aeruginosa (13), Klebsiella pneumoniae (11),Acinetobacter baumanni (2), Candida albicans (1),Escherichia coli (5) and Staphylococcus aureus (1).<5 epithelial cells are Seen in Pseudomonas aeruginosa (12), Klebsiella pneumoniae (14), Candida al- bicans (1), Escherichia coli (3) and Staphylococcus au- reus (1).

Acinetobacter baumanni do not epithelial cells in the category of <5. 5-10 epithelial cells are found in Pseudomonas aeruginosa (7), Klebsiella pneumoniae (7), Acinetobacter baumanni (5) and Escherichia coli (3).

Candida albicans and Staphylococcus aureus do not contain epithelial cells in the category of 5-10. 10-15 epithelial cells are found in Pseudomonas aeruginosa (4), Klebsiella pneumoniae (11), Acinetobacter baumanni (4), Es- cherichia coli (4) and Candida albicans (1). Staphylococcus aureus do not contain epithelial cells in the category of 10-15. 15-20 epithelial cells are seen in Pseudomonas aeruginosa (7), Klebsiella pneumoniae (5), Acinetobacter baumanni (3) and Escherichia coli (7).

Candida albicans and Staphylococcus aureus do not contain epithelial cells in the category of 15-20. 20-25 epithelial cells are found in Pseudomonas aeruginosa (1) and Klebsiella pneumoniae (1). Acinetobacter baumanni, Candida albicans, Escherichia coli and Staphylococcus aureus do not contain epithelial cells in the category of 20-25.

<25 epithelial cells are found in Klebsiella pneumoniae (5), Acinetobacter baumanni (2) and Escherichia coli (1). Pseudomonas aeruginosa, Candida albicans and Staphylococcus aureus do not contain epithelial cells in the category of >25.

CONCLUSION:

1. Most common bacterial isolate from sputum was Pseudomonas aeruginosa.
2. a. The correlation between Direct Gram stained smear Examination and the isolation of bacteria was established, thus making the direct gram stain a useful technique of preliminary examination.
   b. The presence of pus cells between 10 to 25 had a better correlation than that of pus cells less than 10.
   c. The epithelial cells had an inverse correlation with isolation of pathogen and this again brings forth the importance of validation of sputum specimens in a laboratory.
3. These findings help to lay down stringent rejection criteria for sputum samples and also to reiterate the importance of patient education before sample collection
4. The Direct gram smear would help to cut costs of unnecessary sputum processing in a laboratory, in addition to being of paramount importance in presumptive diagnosis of infection before conventional culture results are available, enabling prompt initiation of empirical treatment, in settings where automated AST is not available.

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