Value of Gram Stain as a Predictor of Bacterial Pathogens in Endotracheal Aspirates and Bronchoalveolar Lavage Specimens



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INTRODUCTION

The role of Gram stain to guide therapy for patients with suspected hospital onset and ventilator associated pneumonia is unclear. This study evaluated correlation of Gram stain of endotracheal aspirates (ETA) and bronchoalveolar lavage (BAL) specimens with culture of a potential pathogen (PP) for 466 patient specimens across 4 laboratories.

METHODS

232 ETAs and 234 BALs were tested by Gram stain and culture (**Figure 1**) according to local laboratory standard operating procedures.





Figure 1: a) Gram stain slide; b) Culture plate

RESULTS

• 260 (55.8%) specimens were negative for a PP: 35.8% negative for any growth, and 19.9% with normal respiratory flora.

RESULTS

- 206 (44.2%) specimens were positive for one or more PP, including:
 - Klebsiella spp. (40/206; 19.4%)
 - Staphylococcus aureus (37/206; 17.9%)
 - Pseudomonas aeruginosa (28/206; 13.5%)
 - Candida albicans (20/206; 9.7%)
 - Enterobacter spp. (12/206, 5.8%)
 - Streptococcus pneumoniae (8/206, 3.8%)
- Of the 206 positive specimens:
 - 153/206 (74%) yielded 1 PP
 - 46/206 (22.3%) yielded 2 PPs
 - 5/206 (2.4%) yielded 3 PPs
 - 1/206 (0.4%) yielded 4 and 5 PPs, respectively.
 - The most prevalent combination of PP was *P. aeruginosa* and yeast (n=4).
- Correlation of Gram stain with PP is shown in **Table 1**. Sensitivity and specificity of Gram stain was poor, but negative predictive value was >90%.

| Table 1. Performance of Gram stain vs. culture | | | | | | | | | | | | |
|--|----|----|----|-----|----|---------------------------|------------------------|---------------------|---------------------|--|--|--|
| Gram Stain Result | n | TP | FP | TN | FN | Sensitivity % (95% CI) | Specificity % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | | | |
| Gram-Positive Gram-Positive | | | | | | | | | | | | |
| Staphylococcus aureus | 37 | 22 | 72 | 357 | 15 | 59.5 (43.5-73.7) | 83.2 (79.4-86.5) | 23.4 (16.0-32.9) | 96.0 (93.5-97.5) | | | |
| Gram-Positive Cocci | 59 | 36 | 58 | 349 | 23 | 61.0 (48.3- 72.4) | 85.8 (82.0-88.8) | 38.3 (29.1-48.4) | 93.8 (90.9-95.9) | | | |
| Gram-Negative | | | | | | | | | | | | |
| <i>Klebsiella</i> spp. | 40 | 23 | 51 | 375 | 17 | 57.5 (42.2-71.5) | 88.0 (84.6-90.8) | 31.1 (21.7-42.3) | 95.7 (93.2-97.3) | | | |
| Pseudomonas aeruginosa | 28 | 14 | 60 | 378 | 14 | 50.0 (32.6-67.4) | 86.3 (82.8-89.2) | 18.9 (11.6-29.3) | 96.4 (94.1-97.9) | | | |
| Gram-Negative Rods | 95 | 55 | 19 | 352 | 40 | 57.9 (47.9-67.3) | 94.9 (92.1-96.7) | 74.3 (63.4-82.9) | 89.8 (86.4-92.4) | | | |

RESULTS

- Of 78 specimens with a negative Gram stain, culture yielded:
 - 25 gram-negative PPs
 - 16 gram-positive PP
 - 20 yeast
 - 17 polymicrobial with at least 1 PP.
- For specimens negative for gram-positive cocci by Gram stain, 14 yielded growth of *S. aureus* (**Table 2**).
- For specimens negative for gram-negative bacilli by Gram stain, 10 yielded growth of *P. aeruginosa* and 14 yielded growth of *Klebsiella* spp. (**Table 2**).

| Table 2. Prevalent pathogen growth in samples negative by Gram stain | | | | | | | | | | |
|--|----|---------------|-------------|---------------|---------------|--|--|--|--|--|
| Organism | n | Scant (1+) | Few (2+) | Moderate (3+) | Heavy (4+) | | | | | |
| Gram-Positive | | | | | | | | | | |
| Staphyloccocus aureus | 14 | 4 | 4 | 3 | 3 | | | | | |
| Gram-Negative | | | | | | | | | | |
| Klebsiella spp. | 14 | 3 | 2 | 0 | 9 | | | | | |
| Pseudomonas aeruginosa | 10 | 2 | 2 | 4 | 2 | | | | | |

CONCLUSIONS

Gram stain is a poor predictor of the presence of *S. aureus*, *P. aeruginosa* and *Klebsiella* spp. in ETA and BAL cultures. The value of Gram stain is in its negative predictive value across gram-positive and gram-negative organisms. In this study, the majority of specimens were monomicrobial for potential pathogens, with *Klebsiella* spp., *S. aureus* and *P. aeruginosa* as the most commonly isolated PPs. Laboratories continue to identify yeast in these specimens, although they may not be clinically relevant.