PROFICIENCY TESTING SERVICE
AMERICAN ASSOCIATION OF BIOANALYSTS
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PARTICIPANT STATISTICS **THIRD QUADRIMESTER 2019**

GRAM STAIN

	Specimen 11		Specimen 12		Specimen 13		Specimen 14		Specimen 15		
Gram Stain	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**Neg**	**Pos**	**No. of Labs**
	61	4	0	65	55	9	5	60	19	46	65
Flagging		***	***			***	***				

*Due to a lack of participant consensus, Specimen 15 was not evaluated. The intended result ws Positive.

	Specimen 11		Specimen 12		Specimen 13		Specimen 14		Specimen 15	
Morphology	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**	**No.**	**Flagging**
Cocci	17		54		2	***	3	***	51	
Diplococci	39		1	***	2	***	4	***	3	***
No diplococci present	1	***	2		2		2		1	
Coccobacilli	0	***	0	***	11		9		1	***
Rods (Bacilli)	1	***	0	***	41		39		0	***
Streptococci	0	***	1	***	0	***	1	***	2	
Yeast	0	***	0	***	0	***	0	***	0	***
Total Population	**58**		**58**		**58**		**58**		**58**	

	Specimen 11				Specimen 12			
Gram Stain, educational	**Neg**	**Pos**	**Yeast**	**No pathogen found**	**Neg**	**Pos**	**Yeast**	**No pathogen found**
	40	1	0	0	0	40	0	0

	Specimen 11		Specimen 12	
Morphology, educational	**No.**		**No.**	
Cocci	0		0	
Diplococci	0		0	
No diplococci present	1		1	
Coccobacilli	0		0	
Rods (Bacilli)	40		38	
Streptococci	0		0	
Yeast	0		1	
Total Population	**41**		**40**	

Gram Stain #1 –

Gram Stain #1 – Sputum (*Pseudomonas aeruginosa*)

Clinical Presentation:

A 65-year-old woman was assessed in the ED for a 7-day history of diarrhea, a 4-day history of productive cough with pleuritic chest pain that developed over the last 24 hours. She had been healthy previously, denied having any history of fever or chills, but was a smoker with a 30 pack-year history. On physical examination, the patient had a temperature of 37.8°C and a blood pressure of 110/63 mm Hg. Her respiratory rate was 29 breaths/min. Examination of her chest revealed crackles at the bases of both lungs, with rhonchi present in both upper lobes. Examination of a chest radiograph indicated bilateral opacities consistent with bronchopneumonia. The patient had hypoxia with a partial pressure of oxygen of 60 mm Hg and an oxygen saturation of 89% while breathing room air. Twenty-four hours after admission to the hospital, she was transferred to the ICU, where she underwent intubation for worsening hypoxia and respiratory distress. The results of blood and sputum cultures obtained at the time of the patient's admission to the hospital were both positive with the same organism (see attached photomicrograph of sputum Gram stain).

Discussion narrative:

After overnight incubation, the sputum culture grew pure mucoid colonies that were b-hemolytic on the blood agar plate, oxidase-positive (indole-negative) and displayed the pearlescent colony morphotype characteristic of *Pseudomonas aeruginosa*. The identification of *P. aeruginosa* was subsequently confirmed by the laboratory's automated microbial identification system. Tragically, this case involved rapid onset *Pseudomonas aeruginosa* community-acquired pneumonia (CAP) that ultimately proved to be fatal for this patient. In addition to culture confirmation, autopsy revealed extensive bilateral bronchopneumonia with abscess formation and necrosis of the alveolar walls with significant numbers of polymorphonuclear leukocytes admixed with fibrin in the alveolar space in all lobes of the lung.

Pseudomonas species are usually motile, straight or slightly curved, gram-negative rods (GNR), typically arranged in pairs. The presence of cytochrome oxidase in *Pseudomonas* species is a simple biochemical test that can be utilized to differentiate them from Enterobacteriaceae and *Stenotrophomonas*. As in this case, some strains appear mucoid because of the abundance of a polysaccharide capsule; these strains are particularly common in patients with cystic fibrosis (CF), but not exclusive to the CF patient population. In addition, some species produce diffusible pigments (e.g., pyocyanin [blue], pyoverdin [yellow-green], pyorubin [reddish-brown]) that give them a characteristic appearance in culture and simplify the preliminary identification, as well.

While *P. aeruginosa* is a common nosocomial pathogen that often causes pneumonia in hospitalized patients (i.e., typically, these patients have an underlying medical condition or a risk factor for *Pseudomonas* infection), CAP in previously healthy individuals caused by this pathogen can occur as well, albeit rarely. Such cases can often be rapidly progressive and may be fatal, as in the presented case. *P. aeruginosa* carries a notably higher mortality rate than other pneumonia pathogens due to its multiple mechanisms of antibiotic resistance (e.g., expression of a b-lactamase or efflux pumps and downregulation of outer membrane porins). This problem has been magnified in recent years with the emergence of multidrug-resistant pathogens often unharmed by almost all classes of antimicrobials. Patients at high risk for developing infections include neutropenic or immunocompromised patients, cystic fibrosis patients, and burn patients. *P. aeruginosa* is ubiquitous in nature and moist environmental hospital sites (e.g., sinks, toilets, mechanical ventilation, and dialysis equipment). Such exposure risk factors may be easily overlooked; therefore, the history of such exposures should be carefully assessed in each patient presenting with severe CAP. *P. aeruginosa* must be considered in the differential diagnosis for anyone presenting with a rapidly progressive pneumonia, particularly in patients with a history of smoking GNR in the sputum should raise clinical diagnostic suspicion.

Gram Stain #2 –

Gram Stain #2 – Blood Culture (*Corynebacterium* spp.)

Clinical Presentation:

A 67-year-old man with a 3-day history of chest and acute left foot pain and were associated with fatigue and malaise in the last few preceding months. He had an extensive past medical history including being diagnosed with End Stage Renal Disease (ESRD) 18 years ago and being on hemodialysis via a right femoral catheter during the last three years due to multiple complicating medical conditions (e.g., several fistula failures, pericardial effusion, and deep vein thrombosis). The patient's past surgical history included multiple dialysis catheter placements and AV fistula creation. On admission, lab results included a white blood cell count of 9.0×10^3/mL with 71.3% neutrophils. On the third day, the patient had the second of two episodes of low-grade fever with a maximum temperature of 38.1°C. Therefore, blood cultures were drawn from peripheral veins, and the organism that can be seen in the attached image grew from both sets of blood cultures submitted to the laboratory (aerobic bottles only).

Discussion narrative:

Both blood culture sets drawn following the patient's ICU admission were flagged positive by the laboratory's continuously monitoring blood culture system within 24 hours of having been loaded. Characteristic to this organism group, the Gram stain of both of the aerobic bottles revealed pleiomorphic, gram-positive rods with slightly curved and tapered or clubbed ends (as can be seen in the attached image). Upon subculture, the colonies on the blood agar plate were smooth, white and non-hemolytic after overnight incubation. This patient was diagnosed with a *Corynebacterium striatum* bloodstream infection (i.e., confirmed by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF)) attributed to the patient's indwelling catheter for the treatment of his ESRD.

The Corynebacteria are a group of aerobic, gram-positive, catalase-positive, nonsporulating, and generally nonmotile rods. The Corynebacteria are divided into two groups: *Corynebacterium diphtheriae* and nondiphtherial Corynebacteria, collectively referred to as diphtheroids. Typically, when isolated from clinical specimens, nondiphtherial Corynebacteria, such as *C. striatum*, *C. amycolatum*, *C. minutissimum*, *C. xerosis*, and *Corynebacterium freneyi*, for example, were originally thought to be contaminants, as these strains are commonly considered as part of the normal skin flora, particularly in the axillary, rectal, and inguinal regions of hospitalized patients. However, in recent years, they have been reported as emerging opportunistic pathogens in immunocompromised patients with end-stage cancer, hematologic malignancy, and critical condition. Accordingly, there are several reports of *C. striatum* infections including cases of bacteremia, endocarditis, meningitis, pleuropneumonia, osteomyelitis, arthritis, and intrauterine infections.

More than 80 species of Corynebacterium have been identified and more than 50 species have been related to human diseases. In clinical practice settings, different biochemical test systems have been developed to identify *Corynebacterium* species of clinical significance including API Coryne and RapID CB Plus systems. In addition, molecular methodologies have also been utilized with much success (e.g., 16S rRNA gene sequencing, rpoB sequencing, and MALDI-TOF.

Other organisms for which it can be difficult to distinguish between pathogenicity and contamination include *Cutibacterium* (formerly *Propionibacterium*) *acnes*, *Bacillus* species, and coagulase-negative staphylococci. The likelihood of pathogenicity of these potential blood pathogens (including, *Corynebacterium* spp.) is increased if the organism is observed in multiple blood cultures obtained from separate venipunctures. Consequently, diphtheroids cultivated from blood culture can no longer be unilaterally accepted as only a contaminant, as the possibility of endocarditis in high-risk patients (i.e., especially in those with history of exposure to broad-spectrum antibiotics, immunosuppression or with a central venous catheter in place) remains. This case underscores the importance of *Corynebacterium* spp. as emerging nosocomial pathogens, especially in critically ill patients with indwelling devices.