Difficulty of morphological diagnosis in Gram staining

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DESCRIPTION

A 35-day-old female infant with no apparent perinatal events was presented to our hospital due to fever. Her temperature was 39.2°C, and sinus tachycardia was noted; yet, her general condition was good, and she presented no abnormal physical findings. Blood examination revealed leucocytosis ($22 \times 10^9/L$) and an increase in serum C reactive protein levels (45.5 mg/L), and urinalysis revealed a positive leucocyte elastase value (Urinary leucocyte count was not investigated at the time of diagnosis because the test is unavailable in the emergency room). We diagnosed her with urinary tract infection (UTI); however, unexpectedly, urine Gram staining revealed faintly stained, long and thin Gram-negative bacteria; we suspected *Pseudomonas* spp (figure 1).^{1,2} Although we believe that *Pseudomonas* spp rarely cause UTIs in a healthy infant, we initiated ceftazidime for *Pseudomonas aeruginosa*, according to the result of Gram staining. Fever improved 2 days after the initiation of ceftazidime. The result of urine culture was pan-sensitive *Escherichia coli* (figure 2), and we switched the antibiotic to oral cefalexin. The infant was discharged after the antibiotic switch, and no recurrence was observed in the 2 months after discharge.

In the overall diagnosis of UTI, the sensitivity and specificity of Gram staining have been reported as 81.4% and 98.2%, respectively, in infants younger than 3 months.³ The American Association of Pediatrics recommends Gram staining as a companion to urinalysis for the diagnosis of UTI.⁴ However, another advantage of Gram staining, bacteria estimation, is not well evaluated in adults or in

Figure 2 Cultured colony sample: densely stained, almost round-shaped, Gram-negative bacteria were observed.

children. Based on Gram staining, bacteria can be classified into four groups by colour and shape (ie, Gram-positive/negative cocci/bacilli); however, sometimes, bacterial species can be investigated by a detailed observation of morphological characteristics even among the same group. However, this differentiation is generally difficult and needs training and experience. In this case, we mistook *P. aeruginosa* for *Enterobacteriaceae* as the causative organism based on morphological characteristics, which led to unnecessary broad-spectrum antibiotic administration. This lesson does not apply worldwide because other countries commonly use aminoglycosides that cover *P. aeruginosa* for an infant with UTI (This practice is not quite common in Japan). Nevertheless, it is important to keep in mind the possibility of different morphologies observed in Gram staining. In Japan, the Gram staining technique is one of the attainment targets in the paediatric specialist's certificate programme, to promote antimicrobial stewardship. Educational system involving microbiologists has been established in some teaching hospitals. Gram staining is useful for selecting antibiotics if used wisely⁵; however, we

Figure 1 Urine sample. Faintly stained, long and thin Gram-negative bacteria were observed.

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Learning points

- ▶ Bacteria can present differently in situ vs in culture, leading to different diagnoses through examination of morphological characteristics.
- ▶ Careful consideration is required when using Gram staining for selecting empiric antibiotics.

should know the limitation of Gram stain, and exercise caution while interpreting the results.

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