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Short Communication

Pseudo-Outbreak of Gram-Negative Meningitis Resulting from Contaminated Gram Stain Reagents

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Abstract

We report an outbreak of Contaminated Cerebrospinal Fluid (CSF) specimens, which occurred as a result of contaminated Gram stain reagents. In total, 11 CSF samples were processed where Gram-Negative Bacilli (GNB) were visualised but no organisms were cultured. Following scrutiny of CSF processing, the problem was identified as contaminated Gram stain reagents originating at the manufacturing plant. Clinically, the impact of the false-positive CSF Gram stains was the inadvertent receipt of broad-spectrum antibiotics in one patient and a significant additional workload for both medical scientist and clinical microbiology staff to ensure subsequent patients were managed appropriately.

Keywords: Cerebrospinal fluid; Contamination; Gram stain; Meningitis

Pseudo-Outbreak Part One

On Day 1, a CSF sample was obtained from a 44-year old neurosurgical patient with an External Ventricular Drain (EVD) *in-situ*. An intra-operative CSF sample at insertion contained only 5 white cells/μl and Gram stain did not reveal any organisms. The patient became unwell with a temperature of greater than 39°C and a repeat CSF sample revealed a White Cell Count (WCC) of 180/μl, the differential of which comprised 97% polymorphs and 3% other cells. The findings were consistent with Gram-negative EVD-associated ventriculitis and the patient commenced on meropenem 2g tds intravenously pending culture results. Culture was sterile after 48 hours incubation.

On Day 2, GNB were seen on Gram stain of a CSF sample with a WCC of <1/μl from a 46-year-old female patient admitted with diplopia and ataxia, likely due to a Cerebrovascular event. There were no clinical findings consistent with meningitis and following discussion with the duty clinical microbiologist, a decision was made not to start antibiotics at this time. Once again, CSF culture remained sterile.

Later that night, GNB were noted on Gram stain of a clotted CSF sample, obtained from an EVD of a patient with a complex neurosurgical background. The patient was receiving antimicrobials for brain abscesses. A discussion took place with the duty clinical microbiologist and as the patient's condition was unchanged for a number of days, antibiotics were not escalated. Culture remained sterile.

On Day 3, it became clear that there was a cluster of CSF samples where GNB were visualised on Gram stain yet cultures were sterile. Clinical notes for the three patients with positive CSFs were reviewed and a conclusion drawn that in two out of three cases the clinical picture was not consistent with the laboratory findings, raising the possibility of CSF contamination. As the national neurosurgical referral centre this issue posed a particular problem, as there is a higher likelihood in our complex patient population of GNBs in a CSF specimen being clinically significant.

A laboratory senior management team meeting was convened and the stages where CSF samples could potentially become contaminated considered. These stages included sample collection; sample processing within the Biosafety cabinet and during Gram stain performance. The possibility of contamination at sample collection was deemed unlikely as the CSF samples had come from various locations in the hospital and from different clinical services. The sterile specimen containers were inspected and found to be of different batch numbers, which suggested that contamination occurring at a ward level was unlikely.

The Biosafety cabinet was promptly decontaminated and a decision was made to sterilize glass slides with an alcohol wipe prior to Gram staining. Tap water had traditionally been used to wash slides during Gram staining, however this was viewed as a potential source of contamination and therefore subsequent samples were processed instead using sterile water. New bottles of Gram stain reagents, immersion oil and glass slides were opened and put into use.

Despite these measures, four further CSF samples (two on Day 3 and two on Day 4) had GNB visualised on Gram stain. The total of contaminated samples now numbered seven. On Day 4 a series of Gram stains were carried out using blank sterilized glass slides, sterile water, freshly-made decolorized and the newly-opened Gram stain reagents. GNB were seen. At this point it was suspected that the Gram stain reagents were the source of the contamination. Batch acceptance and Internal Quality Control (IQC) records were checked and it was noted that the bottle of safranin was put into use the day preceding the first falsely positive sample and that the Lugol's iodine was first used the same day as the first falsely positive CSF sample. The reagents were inoculated onto blood agar and incubated. There was no growth despite prolonged incubation.

New Gram stain reagents: crystal violet, Lugol's iodine and safranin with new lot numbers were ordered. On Day 5 a further two CSF samples had GNB visualised on Gram stain. Once again, a series

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of Gram stains were performed using blank sterilized glass slides, sterile water and the new Gram stain reagents from a different lot number. GNB were once again visualised. The finding of GNBs from reagents of a new lot number led us to believe that the contamination was occurring at a central location, likely at the manufacturing plant. Gram stain reagents were promptly ordered from a different manufacturer. On receipt, a series of blank glass slides were stained and no organisms visualised. Gram stain was performed on newly-opened bottles of the old reagents and GNB were visualised, confirming again that the reagents were contaminated on arrival in the laboratory. Seven of the nine contaminated CSF samples were re-processed using the new reagents and no organisms visualised on all seven samples. Two samples were insufficient for repeat Gram stain. All nine samples were sterile after five days incubation. Two remaining unopened bottles of Lugol's iodine were returned to the manufacturers for further investigation.

Pseudo-Outbreak Part Two

Once notified, the manufacturers promptly investigated the production processes. They recalled all Lugol's iodine and safranin of the same lot number. Remaining stock of the same lot number was quarantined and repeat quality control was carried out on these retention samples, both internally and by an external independent laboratory. A full audit trail was conducted on manufacturing process: the raw materials used, the suppliers of the raw materials and the production equipment; a Root-Cause Analysis (RCA) was also carried out. The results of the manufacturer's investigations confirmed the finding of GNBs in the two units of Lugol's iodine returned from our institution. No abnormalities were detected however on retention samples tested both internally and independently. The RCA concluded that a single filling line had silicon tubing that contained residual water. This line is used for the dispensing of water from a Reverse Osmosis system (RO water), which is used in all production processes. As the filling line had not been used for several weeks, the conclusion was drawn that biofilm had formed within the tubing. *Pseudomonas fluorescens* was later identified from samples from the tubing. The filling line tubing was disposed of and new filling lines installed. The filling room was completely sterilized and all glass-wear associated with the production of Lugol's iodine destroyed and replaced. A revised Standard Operating Procedure (SOP) was put in place for tube handling and drying during the RO dispensing stage of production. In addition, all staff involved in the production of Gram stain reagents were fully briefed on the situation.

Discussion

Gram-negative meningitis, although uncommon, is associated with significant morbidity and mortality [1]. A previous study at our institution identified 40 episodes of Gram-negative meningitis involving 34 patients over the time period 1998-2004 [2]. Contamination of sterile site specimens with GNBs has previously been described. Clarke et al. reported the presence of *Burkholderia cepacia* on prolonged culture of sterile site specimens where GNB were visualized on Gram stain [3]. Deionized water used in the preparation of the stain was identified as the source of contamination. Similar reports include a pseudo-outbreak of tuberculosis due to a contaminated phenol red solution and an "epidemic" of pseudomeningitis resulting from Gram stain reagent and cytocentrifuge funnel contamination [4,5]. The pseudo-outbreak at our institution, as the national neurosurgical referral centre, was particularly worrying due to the high volume of CSFs processed in the laboratory on a daily basis and the very often complex clinical nature of these patients. It was crucial to try and promptly identify the source of the contamination in order to minimise the potential harm to patients. There was also the added danger that a true Gram-negative meningitis/ ventriculitis may have been dismissed as being part of the pseudo-outbreak resulting in potentially very serious adverse clinical consequences. Close liaison with clinicians helped to minimise unnecessary antibiotic use in patients where GNBs were seen on Gram stain of CSF. Review of the stages involved in the processing of CSF samples resulted in the identification of the source of contamination bringing the outbreak to an abrupt end. As an additional quality control measure since this incident, we now perform Gram stain using a clean glass slide on all newly-opened Gram stain reagents and daily on the reagents in use to ensure sterility and avoid further pseudo-outbreaks.

Conclusion

A potentially serious "outbreak" of Gram-negative meningitis/ ventriculitis was identified as a pseudo-outbreak due to contaminated Gram stain reagents. Through close collaboration between microbiologists, laboratory staff, clinicians and reagent producers, we successfully and promptly identified the source of contamination and put in place measures to prevent its recurrence. Clinically, the impact of the false-positive CSF Gram stains was the inadvertent receipt of broad-spectrum antibiotics in one patient for a total of seven days with no adverse effects. It also resulted in a significant additional workload for both medical scientist and clinical microbiology staff to ensure subsequent patients were managed appropriately. This report highlights the constant need for vigilance in the laboratory in the processing of sterile site specimens particularly where a cluster of uncommon results occur.

References

1. Durand ML, Calderwood SB, Weber DJ, Miller SI, Southwick FS, Caviness VS Jr, et al. Acute bacterial meningitis in adults. A review of 493 episodes. N Engl J Med. 1993; 328: 21-28.

2. O' Neill E, Humphreys H, Phillips J, Smyth EG. Third-generation cephalosporin resistance among Gram-negative bacilli causing meningitis in neurosurgical patients: significant challenges in ensuring effective antibiotic therapy. J Antimicrob Chemother. 2006; 57: 356-359.

3. Clarke SC, McIntyre M. Contamination of crystal violet in the gram stain method. J Clin Pathol. 1995; 48: 92.

4. Shears P, Rhodes LE, Syed Q, Watson J. A pseudo outbreak of tuberculosis. Commun Dis Rep CDR Rev. 1994; 4: 9-10.

5. Southern PM Jr, Colvin DD. Pseudomeningitis again. Association with cytocentrifuge funnel and Gram-stain reagent contamination. Arch Pathol Lab Med. 1996; 120: 456-458.

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