Isolation of *Salmonella enterica* ssp. *diarizonae* in clinical samples: A diagnostic dilemma

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**ABSTRACT**

A twelve years old boy was admitted in a tertiary care hospital in January, 2015 with history of cough with expectoration, chest pain and fever respectively. On examination, all his vital parameters were normal except low grade fever. No abnormality was detected on systemic examination except slightly reduced air entry in right lung fields. His chest X-ray revealed consolidation of right lung fields with blunting of right costo-phrenic angle. A CT scan of chest was immediately performed which revealed collection of fluid in right pleural cavity and pleural tapping revealed frank pus. Subsequently, a chest drain was placed for the drainage of pleural fluid. Pleural fluid was subjected to culture and sensitivity. Two different organisms namely *Salmonella enterica* ssp. *diarizonae* and *Escherichia coli* were identified by VITEK-2 automated system. Keeping in mind the rare possibility of human infection caused by *Salmonella enterica* ssp. *diarizonae*, this isolate was subjected to conventional biochemical identification tests and eventually identified as a different strain of *Escherichia coli*. The patient was successfully treated and discharged from the hospital after one week. To conclude, we would like to emphasize on the fact that identification by automated systems of bacterial isolates in clinical samples as *Salmonella enterica* ssp. *diarizonae* does not necessarily mean that it is the causative organism. Such bacterial isolates must be subjected to conventional biochemical identification methods. Laboratories should consider several possibilities such as specimen contamination, incorrect identification by automated systems and contamination of sheep blood agar before ascertaining pathogenic status to this organism.

**KEYWORDS:** *Salmonella enterica* ssp. *diarizonae*, *Escherichia coli*, VITEK-2.

**INTRODUCTION**

*Salmonella* spp. are documented to be pathogens that cause a spectrum of diseases in humans and animals, including domesticated and wild mammals, reptiles, birds, and insects. *Salmonella* spp. infections are caused by consumption of contaminated food, person-to-person transmission, waterborne transmission and numerous environmental and animal exposures [1].

*S.enterica* ssp. *diarizonae* is one of the less common subspecies of *Salmonella* which like many non-typhoidal salmonellae is mostly found in animal species (commonly reptiles) and only occasionally infects humans [2]. It is becoming increasingly common to keep reptiles as pets and reports of infection caused by *S. enterica* ssp. *diarizonae* are increasing [3]. Even a pseudo-outbreak of *Salmonella enterica* ssp. *diarizonae* infection has been reported in the past [4].

We present a case report which highlights the issue of bacterial isolates obtained from clinical samples being incorrectly reported as *S.enterica* ssp. *diarizonae* by automated identification systems.
CASE REPORT

A twelve years old male child was admitted in a tertiary care hospital in January, 2015 with history of cough and fever since two months and one month respectively. His cough was associated with expectoration which was copious in amount, green in color, not blood tinged and without any positional and diurnal variation. He also had chest pain on right side which was aggravated by coughing. He was on medication (details not available) under the supervision of a local doctor for more than a month prior to hospitalization but did not show any symptomatic improvement. On examination, all his vital parameters were normal except low grade fever of 99.5˚F. Systemic examination did not reveal any abnormality except slightly reduced air entry all over the lung fields on right side. However, the child was not in respiratory distress. His chest X-ray revealed consolidation of right lung fields with blunting of right costo-phrenic angle.

A CT scan of chest was immediately performed which revealed collection of fluid in right pleural cavity and pleural tapping revealed frank pus. Subsequently, a chest drain was placed for the drainage of pleural fluid. The following investigations were also carried out: hemoglobin- 9.4 g%; total leucocyte count- 11,800; differential leucocyte count- neutrophils 79% and lymphocytes 15%. Pleural fluid analysis revealed a cell count of >50,000 cells/cu.mm with predominance of neutrophils and occasional lymphocytes. Pleural fluid was also subjected to culture and sensitivity. Two different types of colonies were obtained on blood and MacConkey agar (both colonies being lactose fermenting). These were subjected to identification and antibiotic susceptibility testing using VITEK-2 automated system (BioMerieux India Pvt. Ltd.).

Pan drug resistant *Salmonella enterica* ssp. *diarizonae* (resistant to amoxicillin/clavulanate, amikacin, cotrimoxazole, cefotaxime, cefuroxime, ceftriaxone, celepime, ciprofloxacin, офloxacin, gentamicin, imipenem, meropenem and piperacillin/tazobactam) and *Escherichia coli* (sensitive to imipenem, meropenem, amikacin, gentamicin and resistant to amoxicillin/clavulanate, cotrimoxazole, cefotaxime, cefuroxime, ceftriaxone, celepime, ciprofloxacin, офloxacin, imipenem, meropenem and piperacillin/tazobactam) were identified.

Keeping in mind the rare possibility of human infection caused by *Salmonella enterica* ssp. *diarizonae*, we subjected this isolate to conventional biochemical identification tests, the results of which were as follows: Catalase test: Positive; Indole test: Positive; Triple Sugar Iron test: Acid slant/Acid butt with gas; Urea hydrolysis test: Negative; Citrate utilization test: Negative; Malonate utilization test: Negative; Methylene red test: Positive; Fermentation of glucose with production of gas, lactose, sucrose and mannitol respectively. These results indicated that the two supposedly different bacterial isolates were in fact two different strains of *Escherichia coli* with different antibiotic susceptibility patterns.

Before the final culture and sensitivity report was prepared, the patient was empirically treated with injection ampicillin- cloxacillin (ampiclo) which was stopped after two days and replaced by tablet linezolid, injection gentamicin and tablet metronidazole for one week. General condition of the patient improved. Chest drainage gradually decreased in volume and the drain was subsequently removed.

DISCUSSION

Arizona group organism was first isolated by Caldwell and Ryerson in 1939. This group has been called by various names such as *Salmonella arizonae*, *Paracolobacter arizonae*, *Arizona arizonae*, *Arizona hinshawii* etc [11]. *S. enterica* ssp. *diarizonae* is part of the normal reptile intestinal flora but can cause disease in monotremes, turkeys, chickens, goats, and rarely in humans [5].

*S. enterica* ssp. *diarizonae* enteritis or systemic infections have been well described in patients resident in the southern states of the USA and Europe it is much rarer, with only a few cases reported in the literature [6-9]. Most cases of invasive *S. enterica* ssp. *diarizonae* infection have been either in younger patients or those with underlying diseases including collagen vascular diseases, malignancy, organ transplantation and HIV infection [10].

It can be difficult to identify *S. enterica* ssp. *diarizonae* due to their distinguishing biochemical features such as the ability to utilize malonate, liquefy gelatin and the inability to grow in the presence of KCN (potassium cyanide), which are generally not looked into for routine identification of bacterial isolates. Isolation of *S. enterica* ssp. *arizonae* from the stools is difficult as some strains ferment lactose within 48 hours (approximately 15%) and they may be routinely discarded as non-pathogens. However the presence of hydrogen sulfide is an important diagnostic clue during routine screening [11].

In our case out of the two organisms (*Escherichia coli* and *Salmonella enterica* ssp. *diarizonae*) isolated in culture of pleural fluid obtained from our patient, *Salmonella enterica* ssp. *diarizonae* was pan-drug resistant. However, *Escherichia coli* was relatively sensitive to certain antibiotics including gentamicin which was eventually used (as one of the drugs) to successfully treat this patient. Isolation of *Salmonella enterica* ssp. *diarizonae* from pleural fluid probably occurred as a result of misidentification by VITEK-2 system as this isolate was later correctly identified as *Escherichia coli* using conventional biochemical identification tests [12]. Similar observation was made a month later when an isolate from spumum sample of another patient admitted in our hospital was incorrectly identified as *Salmonella enterica* ssp. *diarizonae*. VITEK-2 sytem cannot be completely relied upon for identification of Gram negative rods. In a study conducted by Guido Funke et al, 84.7% of the isolates of Gram negative bacilli (either belonging to the family Enterobacteriaceae or otherwise) were correctly identified at the species level [13].

Isolation of unusual bacteria from clinical specimens might sound an alarm regarding a likely outbreak keeping in mind the background prevalence and pattern of bacterial isolates in hospital settings. Thiolet et al had reported pseudo-outbreak of *Salmonella enterica* ssp. *diarizonae* infection in France in 2008. The authors had successfully demonstrated that the rare *SIIIB* serotype 61:k:1,5,7 or its monophagic variant 61:--;1,5,7 considered to be adapted to sheep, may


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cause sheep blood agar contamination and lead to false-positive culture results and to pseudo-outbreaks [4].

Concomitant isolation of *Salmonella enterica* subspp. *diarizonae* from pleural fluid, stool and blood samples obtained from our patient would have pointed towards this organism being the most likely pathogen. However, since stool and blood samples were not sent for culture, therefore, we cannot correlate that *Salmonella enterica* subspp. *diarizonae* was the causative organism. This isolate was pan-drug resistant but the patient responded to antibiotic therapy. Also, the pleural fluid sample was obtained from a drainage tube. All these findings indicate that *Salmonella enterica* subspp. *diarizonae* was most probably a contaminant.

**CONCLUSION**

To conclude, we would like to emphasize on the fact that identification (by automated systems) of bacterial isolates in clinical samples as *Salmonella enterica* subspp. *diarizonae* does not necessarily mean that it is the causative organism. Such bacterial isolates must be subjected to conventional biochemical identification methods. Concomitant isolation of this organism from different clinical samples obtained from the same patient and clinical history should be considered before reaching any conclusion. Laboratories should consider several possibilities such as specimen contamination, incorrect identification by automated systems and contamination of sheep blood agar with *Salmonella enterica* subspp. *diarizonae* in case the aforementioned criteria of ascertaining the pathogenic status of this organism are not met.

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**REFERENCES**


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