

## International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693  
 ISSN Online: 2617-4707  
 IJABR 2024; SP-8(3): 694-696  
[www.biochemjournal.com](http://www.biochemjournal.com)  
 Received: 09-01-2024  
 Accepted: 17-02-2024

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## Identification of opportunistic bacteria in buffalo species suffered with gastrointestinal tract disorders

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DOI: <https://doi.org/10.33545/26174693.2024.v8.i3Si.865>

### Abstract

The present study was conducted on 38 buffalo carcasses (17 adult buffalo and 21 buffalo calves) affected by gastrointestinal tract disorders received for post-mortem examination at the Department of Veterinary Pathology, LUVAS, Hisar, during a period of August 2019 to January 2020. Bacteriological isolations were carried out aseptically from the heart blood, liver, and mesenteric lymph nodes. Identification of bacterial species through the *Vitek-2* Compact system revealed that most of the bacterial species were identified in the liver, followed by mesenteric lymph nodes and heart blood. *Escherichia coli* were found to be the most prevalent bacterial species in mesenteric lymph nodes, followed by liver and heart blood.

**Keywords:** Opportunistic, gastrointestinal, vitek-2, Gram positive and Gram negative

### Introduction

In recent years, new emerging opportunistic bacteria have become major problems for livestock and human health. In healthy hosts, the commensal microorganisms contribute colonization resistance, which prevents the expansion of the pathogens, but in immunocompromised hosts, due to disruption of the beneficial commensal microorganisms, the pathogens adopt and gain an advantage to grow and multiply (Wu Y. *et al.*, 2021) [1]. *Escherichia coli* are the predominant non-pathogenic microorganisms in the human intestine (Nataro and Kaper, 1998) [2]. The resident microorganisms in the gut mucosa are very useful and necessary for the hosts to exclude the exogenous pathogens (Kamada and Nunez, 2014) [3], but in certain circumstances, they may be exploited by the pathogens like *Bacteroides spp.*, which comprise a predominant gene in the intestinal tract that promotes the expression and replication of the virulence genes of *Escherichia coli* (Baumler and Sperandio, 2016) [4]. Likewise, *Staphylococcus aureus*, due to the secretion of  $\alpha$ -toxin, can prevent itself from being cleared by the macrophages, resulting in the expansion of the opportunistic pathogens. The present study focused on the identification of the new emerging opportunistic bacterial species.

### Materials and Methods

#### Sample collection and processing

A total of 114 samples were collected from 38 buffalo species (17 adult and 21 buffalo calves) carcasses suspected of gastrointestinal tract disorders brought to the Department of Veterinary Pathology, LUVAS, Hisar for post-mortem examinations from August 2019 to January 2020. The samples were collected in aseptic conditions from the heart blood, liver, and mesenteric lymph nodes of the buffalo carcasses, which showed gross lesions of disorders in the gastrointestinal tract.

#### Isolation of bacteria

The samples were streaked on nutrient agar (NA), Mac Conkey's Lactose Agar (MLA), eosin-methylene blue agar (EMB), and xylose-lysine deoxycholate agar (XLD) for the isolation of the bacteria. The inoculated plates were incubated at 37°C for 24 hours. After incubation, the plates were examined for the presence of any bacterial growth and observed for characteristic features of different types of bacterial colonies so obtained on agar plates

(Cruickshank, 1975) [5], and then the individual colonies were subjected to Gram's staining. Then the isolates were selected for further characterization and confirmation by the Vitek-2 Compact system.

### Identification of bacteria through Vitek-2 Compact system

Gram positive and Gram-negative reagent cards with 64 wells containing individual test substrates were used. With the help of a sterile swab or applicator, a sufficient number of colonies from the pure culture were transferred into 12\*75 mm clear plastic (polystyrene) test tubes containing 3.0 ml of sterile saline solution (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0). The turbidity was adjusted accordingly and measured by a turbidity meter called Densi Chek TM, and then the test tubes containing suspension were placed in a special rack (cassette). After that, the cassettes with test tubes and reagent cards were loaded into the Vitek-2 Compact system. The results were interpreted as per the instructions of the manufacturer (Bio Merieux, Inc., Hazelwood, MO, USA).

### Results and Discussion

The current study started from August 2019 to January 2020, and as per the owner's history, most of the animals died suddenly with no major clinical signs or symptoms. Some animals were treated for gastrointestinal disorders like diarrhoea, etc. The microbiological studies revealed that out of 38 animals, 26 animals (13 adult buffaloes and 13 buffalo calves) were found positive for bacteriological isolation, and a total of 42 bacterial isolates were obtained. The colony characteristics on nutrient agar revealed smooth, circular white-greyish white colonies in 15 isolates, golden yellow colonies in 4 isolates, smooth circular creamy-pinkish colonies in 11 isolates, and colonies with swarming growth in one isolate. Colonies on MLA revealed pale colonies in 15 isolates, which were further streaked on EMB agar, and smooth, circular, greenish-black colonies with a metallic sheen appeared after 24 hours of incubation. On XLD agar, a red colony with a black center appeared in one isolate. Out of 42 isolates, the vitek-2 compact system identified 19 different species of bacteria.

The different identified bacterial species are depicted in Table 1. Out of 42 different species of bacteria, *Escherichia coli* was identified as the most prevalent bacterial species (35.71%), followed by *Sphingomonas paucimobilis* (9.52%), *Pseudomonas mendocina* (7.14%), *Enterococcus faecalis* (4.76%), *Kocuria rosea* (4.76%), *Serratia plymuthica* (4.76%), *Stenotrophomonas multophila* (4.76%), *Kocuria kristinae* (2.38%), *Gardnerella vaginalis* (2.38%), *Salmonella spp.* (2.38%), *Ochrobacterum anthropi* (2.38%), *Proteus mirabilis* (2.38%), *Staphylococcus warneri* (2.38%), *Staphylococcus haemolyticus* (2.38%), *Staphylococcus lentus* (2.38%), *Streptococcus uberis* (2.38%), *Streptococcus alactolyticus* (2.38%), *Micrococcus luteus* (2.38%), and *Pseudomonas aeruginosa* (2.38%). The maximum number of *Escherichia coli* was also reported in bovine previously by Lehreena *et al.* (2010), [6] Londhe (2010) [7] and Sushma *et al.* (2016) [8], along with *Proteus mirabilis* and *Staphylococcus spp.* In addition to the above bacterial species, *Salmonella spp.* was also reported by Londhe (2010) [7] and Sushma *et al.* (2016) [8]. Borai *et al.* (2013) [9] also reported the isolation and identification of

*Escherichia coli*, *Pseudomonas spp.*, *Streptococcus spp.*, *Staphylococcus spp.*, and *Proteus spp.* from the liver of ruminants. In our investigations, *Kocuria spp.* and *Sphingomonas paucimobilis* were identified in 7.14% and 9.52% of cases, respectively. These two species of bacteria were also isolated in the study of Al-Traif *et al.* (2018) [10] from small ruminants. *Kocuria spp.* belongs to the *Micrococcaceae* family, which also includes *Staphylococcus spp.* and *Micrococcus spp.* Likewise, *Ochrobacterum anthropi* belongs to the *Brucellaceae* family. Most of the newly identified bacterial species belong to opportunistic bacteria and are responsible for infection in suppressed and immunocompromised animals, including human beings. *Kocuria spp.* is involved in many of these conditions, which were also isolated by many reporters previously, like Masouf *et al.* (2009) [11] in urinary tract infections, Tsai *et al.* (2010) [12] in brain abscess, Lee *et al.* (2009) [13] in peritonitis and Mattern and Ding (2014) [14] in keratitis cases. Other such opportunistic bacteria were also previously isolated, like *Stenotrophomonas multophila*, which is also an environmental multiple drug-resistant pathogen and involved in many infections like pneumonia (Fujita *et al.*, 1996) [15], biliary sepsis (Papadakis *et al.*, 1995) [16], urinary tract infections (Abdulhak *et al.*, 2009) [17], and eye infections, i.e., keratitis and scleritis (Lin *et al.*, 2011) [18]. Cengiz *et al.* (2015) [19] reported the isolation and identification of *Sphingomonas paucimobilis* in animals suffering from pneumonia and septicaemia. Like other opportunistic bacteria, *Ochrobacterum anthropi* is involved in many infections, like endocarditis (Gomez *et al.*, 2004) [20] and meningitis (Chang *et al.*, 1996) [21].

**Table 1:** Bacterial species identified from buffalo species

Sn	Bacterial spp. Identified	Organs				
		Liver	MLN	HB	Total	%age
1	<i>E. coli</i>	5	7	3	15	35.71
2	<i>Enterococcus faecalis</i>	1	1	0	2	9.52
3	<i>Kocuria rosea</i>	2	0	0	2	7.14
4	<i>Kocuria kristinae</i>	1	0	0	1	4.76
5	<i>Gardnerella vaginalis</i>	0	0	1	1	4.76
6	<i>Sphingomonas paucimobilis</i>	3	0	1	4	4.76
7	<i>Salmonella group</i>	0	1	0	1	4.76
8	<i>Ochrobacterum anthropi</i>	0	1	0	1	2.38
9	<i>Proteus mirabilis</i>	1	0	0	1	2.38
10	<i>Staphylococcus warneri</i>	1	0	0	1	2.38
11	<i>Staphylococcus haemolyticus</i>	0	0	1	1	2.38
12	<i>Staphylococcus lentus</i>	0	0	1	1	2.38
13	<i>Streptococcus uberis</i>	0	1	0	1	2.38
14	<i>Streptococcus alactolyticus</i>	0	0	1	1	2.38
15	<i>Serratia spp.</i>	2	0	0	2	2.38
16	<i>Stenotrophomonas multophilla</i>	1	0	1	2	2.38
17	<i>Micrococcus luteus</i>	1	0	0	1	2.38
18	<i>Pseudomonas mendocina</i>	2	0	1	3	2.38
19	<i>Pseudomonas aeruginosa</i>	0	1	0	1	2.38
	Total	20	12	10	42	100

### Conclusion

Most of the newly identified bacteria belong to opportunistic pathogens. These bacteria could easily be accessed by humans through the food chain. Rapid and easy identification of bacteria is now necessary. The Vitek-2 compact system helps in the rapid identification of bacterial species.

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