Isolation & antibiotic susceptibility pattern of *Shigella* spp.isolated from food & water samples

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Abstract

the global burden of foodborne infections has risen significantly, with nearly one-quarter of the population now considered at high risk. Food, while essential to human well-being, is also a common source of anxiety due to potential contamination due to various pathogenic bacteria Shigella species are of particular concern.In the study a total of 40 samples of Peda (10) Regula (10), Milk (10) and Drinking water (10) were collected from the various sites and shops of Akola. The overall high prevalence of 82.5% (33 out of 40 samples testing positive) indicates widespread Shigella contamination in the region, with significant risks associated with both food and water sources. The varying levels of antibiotic resistance among Shigella isolates where Gentamicin (90.9% sensitivity) and Ciprofloxacin (75.75% sensitivity) remained relatively effective, other antibiotics like Tetracycline, Ampicillin, and Amoxiclav showed high resistance rates.Enhanced surveillance, sanitation measures, and public health education are necessary to prevent the potential spread and outbreaks.

KeyWords- *Shigella* spp.**,**foodborne, antibiotic resistance

Introduction

Over the last 25 years, the global incidence of foodborne infections has markedly increased, with nearly a quarter of the population at a high risk of illness (Oliver *et al., 2005)*. Food greatly contributes to human well-being and is a major source of anxiety, pleasures, and stress. Food is regarded safe and hygienic when there is no dangerous substance that can be injurious to health. Foods and drinks can be contaminated, thereby becoming inappropriate for consumption. Food contamination, either from microbiological or chemical origin, is a major concern for consumers. It can cause catastrophic health and economic effects. Food-borne illnesses cause major international health problems and reduced economic growth. Food contamination incidents, whether intentional or unintentional, can have dire consequences for both the customer who eats it and the business who sold it. (Sadiku *et. al., 2020*)

Food contamination occurs when bacteria or other germs get into food that should not be there, thereby making the food unsafe for consumption. It may also be regarded as foods that are spoiled because they contain microorganisms that make them unfit for consumption. Biological Contamination: This occurs when bacteria, fungal, or other harmful microorganisms contaminate food. It is a common cause of food poisoning, food spoilage, and food-borne illness. Although all foods can harbor dangerous pathogens, some foods are more vulnerable to biological contamination than others some highly contaminated.

In addition, water borne Pathogenic microorganisms are introduced into The water system through research laboratories, Hospitals, untreated sewage, septic tanks, Tanneries, food processing facilities, and meat

(Schwarzenbach,*.et al.,2010*). The lack of safe Water, poor hygiene, close personal contact and malnutrition Play an important role in the transmission of *Shigella* (Torres AG Rev Latinoam Microbiol 2004) Shigellosis is a global human health problem and major cause of diarrhoea causing about 700.000 deaths per year worldwide (Chattaway MA, *et.,al*.2017) Due to improper care, shigellosis may lead to a life-threatening systemic disease known as a hemolytic uremic syndrome, characterized by hemolytic uremia, thrombocytopenia, and kidney failure. Approximately 2-3% of *S. flexneri* infected individuals may develop eye irritation, pain during urination, and joint pains (Singh.et.,al.1985)

Shigella species are non-spore forming,non-motile gram negative.oxidase negative, facultative anaerobic bacteria. Their colonies are convex and round and have intact edges. The colonies have a diameter of about 2mm within 24 hours (SA, Mietzner TA.Medical Microbiology.) It Is a non-motile, facultatively anaerobic, rod-shaped, gram-negative bactę-ria and is associated with bacterial species belonging to the Enterobacteriaceae family Which are mainly responsible for causing. Bacillary dysentery. In India (eastern India), *S. flexneri* (60.7%) is the most prevalent one, followed by *S. sonnei* (23.8%). *S. dysenteriae* (9.8%), and finally *S. boydii* (5.7%) serogroup. Interestingly in South India, *S. flexneri* (> 90%) is found again to be the most prevalent one, followed by *S. sonnei* 3.9-5.4% (Taneja N, mewaara A 2016) It Is gram negative bacilli, non-spore forming, non-motile, 0.5-0.7 µm in size and facultative Anaerobic pathogen that are closely related to *Escherichia coli.*

Different antibiotics (such as a fluoroquinolone, sulfamethoxazole, ampicillin, ciprofloxacin, and nalidixic acid) are prescribed for The treatment of shigellosis. But, the increasing number of Antibiotic-resistant bacteria is becoming a global problem (Sivapalasingam.s.*et.,al.*1999 to 2002) Antibiotics are helpful for the treatment of shigellosis. Although diarrhoea caused by *S. sonnei* is generally mild and self-limited in healthy adults in industrialised countries, Infection with any *Shigella* species can be lethal to children in developing countries. This Is particularly true in the very young, malnourished or immuno-compromised patients. Without effective antibiotic treatment, mortality due to *Shigella* infection, especially from Infection with *S. dysenteriae* type 1 may exceed 10%, particularly among the young and thebelderly (Bennish and Wojtyniak 1991) Because of increasing concerns about emergence of drug resistance and side-effects, other Kind of antimicrobial agents have been investigated. Rifaximin, a non-absorbed rifamycin Derivative, has been shown to be effective in preventing shigellosis in a volunteers Challenge study (Taylor *et al.* 2006) There are no human clinical trials to evaluate this drug in the treatment of *Shigella* infection. A diet with green banana, which is rich in amylase-resistant starch that stimulates colonic production of short-chain fatty-acid, has been reported improving the clinical severity of childhood shigellosis treated with ciprofloxacin (Rabbani *et al.* 2009)

Material and Methods

*** Collection of samples:**

A total of 40 samples of Peda (10) Regula (10), Milk (10) and Drinking water (10) were collected from the various sites and shops of Akola. The samples were collected in sterilized Containers and transported in ice bag containers within one hour into laboratory for further work.

*** Enrichment of samples using Selenite broth:**

The sample collected were then inoculated into sterilized selenite F broth and kept for Incubation at 37°C for 18-24hrs. After incubation the samples from enrichment medium were used for isolation of *Shigella* Species.

*** Isolation and identification of *Shigella* species:**

The samples from enrichment broth were Inoculated onto the Sterilized *Salmonella Shigella* (SS) agar and Xylose Lysine Deoxycholate (XLD) agar. The plates were incubated at 37°C for 24 hrs. After incubation the pure

colonies were observed were picked and inoculated onto the nutrient agar slant and pure cultures were maintained at 4ºC. The pure cultures were then used for identification using Standard microbiological methods including cultural, microbiological, and biochemical characteristics.

***Determination of Antibiotic Susceptibility testing of isolates:**

Antimicrobial susceptibility of *Shigella* species was determined by the disc diffusion method in accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI). The antibiotics used were ciprofloxacin (5µg), gentamicin (10µg), tetracycline (10µg), ampicillin (30µg), vancomycin (10µg), amoxicillin (30µg). In this the Muller Hinton agar was prepared and sterilized by autoclaving at 121ºC for 15 min. The media was poured into sterile petri plates. After solidification the broth cultures of test isolates were uniformly spreaded. With the help of sterile forcep each antibiotic disc was placed at equidistance. The plates were then incubated at 37ºC for 24 hrs. After incubation the zone of inhibition observed were measures and resistance/ susceptibility of isolates were determined.

Results and Discussion-

The study provides valuable insights into the prevalence, characteristics, and antibiotic resistance patterns of Shigella species in food and water sources in the Akola region. The results from the various sample types, as well as the characteristics of the isolates, reveal important information that can help in understanding the scope of Shigella contamination and in formulating strategies for public health intervention.

In the present study total 40 samples viz. Peda,milk,rasgulla and drinking water was collected.10 samples of different types collected from various sites.as in Peda samples were: Collected from places like sweet marts, hotels, and road areas..Milk samples Mostly from dairies spread across the region.Rasgulla from in sweet marts and dairies.while Drinking Water taken from common public places such as bus stands, hospitals, and local roads (table 1).

Amongst Peda, 8 sites Rathi Pedevala, Jatharpeth:Shri Shivaji College Road, Ashok Sweet:Bhirad Hotel, Dabki Road:Murli Sweet, Dabki Road:Nirmal Sweet, Gajanan Nagar:Tripathi Sweet, Akot Fail:Aayesha Sweet, Firdos Colony: Gandhi Road, Nirmal Sweet showed Presence of Shigella, while 2 sites Gujrati Sweet Mart, Railway Station: Shri Gurukrupa Sweet, Gorakshan Road

Among Milk samples all 10 samples collected from various sites showed presence of *Shigella*

In Rasgulla 7 samples showed presence of Shigella from sites Yashoda Sweet Mart, Choti Umri, Shri Ganesh Dairy, Mothi umri, Murli Sweet Mart, Dabki Road, Nirmal Sweet Mart, Dabki Road, Mitali Dudh Dairy, Jawahar Nagar Rd, Tilak Rd Akola, Khatri Namkin, Tripathi Sweet, Akot Fail. While 3 sites Shri Balaji Sweet Mart, Barshitakli Rd, Mahakali Sweet, Murtijapur Rd Kirti Nagar, Hotel City Pride, Railway Station Rd showed no presence of test pathogen.

Among Drinking Water samples collected, from Area of Bus Stand, Akola, Shri Shivaji College, Akola,Rawankar Hospital, Akola,Akot Phail, Akola, Khadan, Akola,Choti Umri, Akola,Gorakshan Road, Akola showed occurrence of Shigella.while samplea from area of Tarphail, Akola, Bhim Nagar, Akola, Nimbuni Nagar Railway Gate, Akola were found free from shigella (table 2).

The overall prevalence rate of *Shigella* species in the samples was found to be 82.5%, with 33 out of 40 samples testing positive. This high prevalence is concerning as it suggests widespread contamination in both food and water sources across the Akola region, indicating a significant public health risk (table3).

In the study, 90% of the peda samples tested positive for Shigella. This is a high contamination rate, particularly concerning as sweet foods are widely consumed. The positive sites included popular sweet marts and local shops of city. Contamination may stem from unhygienic preparation or storage conditions, emphasizing the need for better sanitation and hygiene practices in local food businesses.

100% of milk samples tested positive for Shigella, highlighting milk as a major vehicle for pathogen transmission. Since milk is consumed widely and often in raw or minimally processed forms, its contamination is a major concern. This suggests that dairy farms or dairies in Akola might have significant hygiene issues, and proper handling, pasteurization, or treatment of milk could be critical to reducing Shigella contamination.

While 70% of rasgulla samples tested positive for Shigella, which is a moderate contamination rate. This highlights that contamination is not only prevalent in peda but also in other sweet products like rasgulla. Although the contamination rate is lower than that for milk or peda, it still represents a health risk, particularly since these sweets are consumed in various public settings.

Among Drinking Water Samples:, 70% of samples tested positive for Shigella, indicating that water contamination is a major concern in Akola. Water sources like Bus Stand, Shri Shivaji College, and Rawankar Hospital were all contaminated.Contaminated water sources can act as a vehicle for the spread of Shigella, further highlighting the need for improved water treatment and monitoring of water quality in public areas(Graph 1)

The biochemical and morphological characteristics of the Shigella isolates were consistent with common features of the genus:.Overall, the isolates exhibited classic Shigella characteristics, with the identification of Shigella dysenteriae, Shigella boydii, and *Shigella* sonnei based on the biochemical and cultural tests (table4).

In the study antibiotic susceptibility pattern of isolates was also determined(Graph 2). The antibiotic resistance profile of the *Shigella* isolates reveals critical findings for the treatment of infections caused by these pathogens. It was found that 75.75% of isolates were sensitive to Ciprofloxacin, making it an effective treatment for the majority of cases. However, the 24.25% resistance rate suggests that antibiotic resistance is still a concern, and regular monitoring of resistance patterns is necessary

90.9% of isolates were sensitive to Gentamicin, . Only 9.1% of isolates were resistant, which is a relatively low resistance rate. To the tetracycline 60.67% of isolates were resistant , making it less effective in the region. This highlights the need for alternative therapies, as Tetracycline resistance is widespread. The high resistance rate of 78.8% to Ampicillin shows that this antibiotic is largely ineffective for treating Shigella infections in the study area. About 81.8% of isolates were sensitive to Vancomycin, indicating moderate effectiveness, though some resistance exists (18.2%). 69.65% of isolates were resistant to Amoxiclav, showing that it is not a reliable treatment for *Shigella* infections in this region.

Overall Gentamicin and Ciprofloxacin are the most effective antibiotics, with high sensitivity rates (90.9% and 75.75%, respectively). Tetracycline, Ampicillin, and Amoxiclav show high resistance rates (60.67%, 78.8%, and 69.65%, respectively), suggesting that these antibiotics may be less effective in treating Shigella infections in the study region. While Vancomycin has moderate effectiveness, with an 81.8% sensitivity rate and relatively lower resistance. (Graph No 2)

Study revealed varying levels of antibiotic resistance among *Shigella* isolates. While Gentamicin (90.9% sensitivity) and Ciprofloxacin (75.75% sensitivity) remained relatively effective, other antibiotics like Tetracycline, Ampicillin, and Amoxiclav showed high resistance rates, limiting therapeutic options.The high resistance rates to common antibiotics emphasize the need for alternative treatments and the importance of monitoring antibiotic resistance to ensure the effective management of *Shigella* infections in the region.

The widespread contamination of milk, sweets, and drinking water points to significant gaps in sanitation, hygiene, and food safety practices across various sectors in Akola. Immediate improvements in food handling, water treatment, and antibiotic stewardship are essential to prevent further spread and protect public health.

Shigella contamination is a serious public health concern, particularly due to its high prevalence and the potential for outbreaks. Enhanced surveillance, sanitation measures, and public health education are necessary to curb the spread of this pathogen.

Table 1: collection of samples from various sites of Akola region.

Sr no	Type of sample	Site of collection
1	Peda	Rathi pedevala, Jatharpeth
2	Peda	Shri Shivaji college road, ashok sweet
3	Peda	Bhirad hotel, dabki road
4	Peda	Murli Sweet, Dabki road
5	Peda	Nirmal sweet, Gajanan nagar
6	Peda	Gujrati Sweet Mart, Railway station
7	Peda	Tripathi sweet, Akot fail
8	Peda	Aayesha sweet, Firdos colony
9	Peda	Gandhi road, Nirmal sweet
10	Peda	Shri gurukrupa sweet, Gorakshan road
11	Milk	Krusna dairy, Mohhamad ali road
12	Milk	Journal distributor, Gadanki
13	Milk	Journal distributor, Chandur
14	Milk	Sisodiya dairy, Bhavani peth
15	Milk	Gurukrupa dairy, Dabki road
16	Milk	Shri rajrajeshvar dairy, Dabki road
17	Milk	Gurukrupa dairy, Jatharpeth nakshtra apnt
18	Milk	Shri krishna dairy, Kapila nagar road
19	Milk	Shri gajanan dairy, Gandhi gram
20	Milk	Patil dudh dairy, Bypass Akola
21	Rasgulla	Yashoda Sweet Mart, Choti umri
22	Rasgulla	Shri ganesh dairy, Mothi mri
23	Rasgulla	Murli Sweet Mart, Dabki road
24	Rasgulla	Nirmal Sweet Mart, Dabki road
25	Rasgulla	Mitali dudh dairy, Jawahar nagar rd
26	Rasgulla	Shri balaji Sweet Mart, Barshitakli rd
27	Rasgulla	Tilak rd Akola, khatri namkin
28	Rasgulla	Mahakali sweet, murtijapur rd kirti nagar

29	Rasgulla	Hotel city pride, Railway station rd
30	Rusgulla	Akot fail, Tripathi sweet
31	Drinking water	Bus sttand Akola
32	Drinking water	Shri Shivaji college Akola
33	Drinking water	Rawankar hospital Akola
34	Drinking water	Tarphail Akola
35	Drinking water	Bhim nagar Akola
36	Drinking water	Akot phail Akola
37	Drinking water	Khadan Akola
38	Drinking water	Nimbuni nagar railway get Akola
39	Drinking water	Choti umri Akola
40	Drinking water	Gorakshan road Akola

Table 2: Isolation of *Shigella* species from various sample collected from various sites

Sr no	Type of sample	Site of collection	Presence of *Shigella* species
1	Peda	Rathi pedevala, Jatharpeth	+
2	Peda	Shri Shivaji college road, ashok sweet	+
3	Peda	Bhirad hotel, dabki road	+
4	Peda	Murli Sweet, Dabki road	+
5	Peda	Nirmal sweet, Gajanan nagar	+
6	Peda	Gujrati Sweet Mart, Railway station	-
7	Peda	Tripathi sweet, Akot fail	+
8	Peda	Aayesha sweet, Firdos colony	+
9	Peda	Gandhi road, Nirmal sweet	+
10	Peda	Shri gurukrupa sweet, Gorakshan road	+
11	Milk	Krusna dairy, Mohhamad ali road	+
12	Milk	Journal distributor, Gadanki	+
13	Milk	Journal distributor, Chandur	+
14	Milk	Sisodiya dairy, Bhavani peth	+
15	Milk	Gurukrupa dairy, Dabki road	+
16	Milk	Shri rajrajeshvar dairy, Dabki road	+
17	Milk	Gurukrupa dairy, Jatharpeth nakshtra apnt	+
18	Milk	Shri krishna dairy, Kapila nagar road	+
19	Milk	Shri gajanan dairy, Gandhi gram	+
20	Milk	Patil dudh dairy, Bypass Akola	+
21	Rasgulla	Yashoda Sweet Mart, Choti umri	+
22	Rasgulla	Shri ganesh dairy, Mothi mri	+
23	Rasgulla	Murli Sweet Mart, Dabki road	+
24	Rasgulla	Nirmal Sweet Mart, Dabki road	+
25	Rasgulla	Mitali dudh dairy, Jawahar nagar rd	+
26	Rasgulla	Shri balaji Sweet Mart, Barshitakli rd	-
27	Rasgulla	Tilak rd Akola, khatri namkin	+

28	Rasgulla	Mahakali sweet, murtijapur rd kirti nagar	-
29	Rasgulla	Hotel city pride, Railway station rd	-
30	Rasgulla	Tripathi sweet, Akot fail	+
31	Drinking water	Bus sttand Akola	+
32	Drinking water	Shri Shivaji college Akola	+
33	Drinking water	Rawankar hospital Akola	+
34	Drinking water	Tarphail Akola	-
35	Drinking water	Bhim nagar Akola	-
36	Drinking water	Akot phail Akola	+
37	Drinking water	Khadan Akola	+
38	Drinking water	Nimbuni nagar railway get Akola	-
39	Drinking water	Choti umri Akola	+
40	Drinking water	Gorakshan road Akola	+

Table 3: Prevalence of *Shigella* species from food and water samples

Sample	No of samples	Occurrence *Shigella* species	Percentage (%)
Peda	10	9	90
Milk	10	10	100
Rasgulla	10	7	70
Water	10	7	70
Total	40	33	82.5

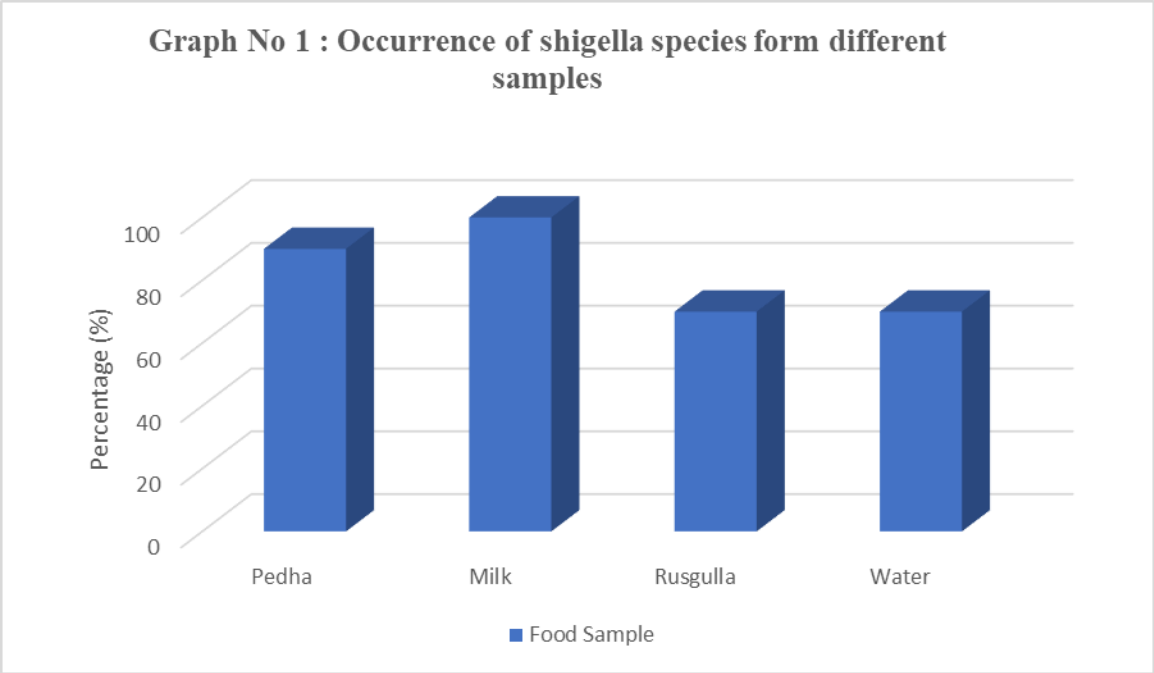
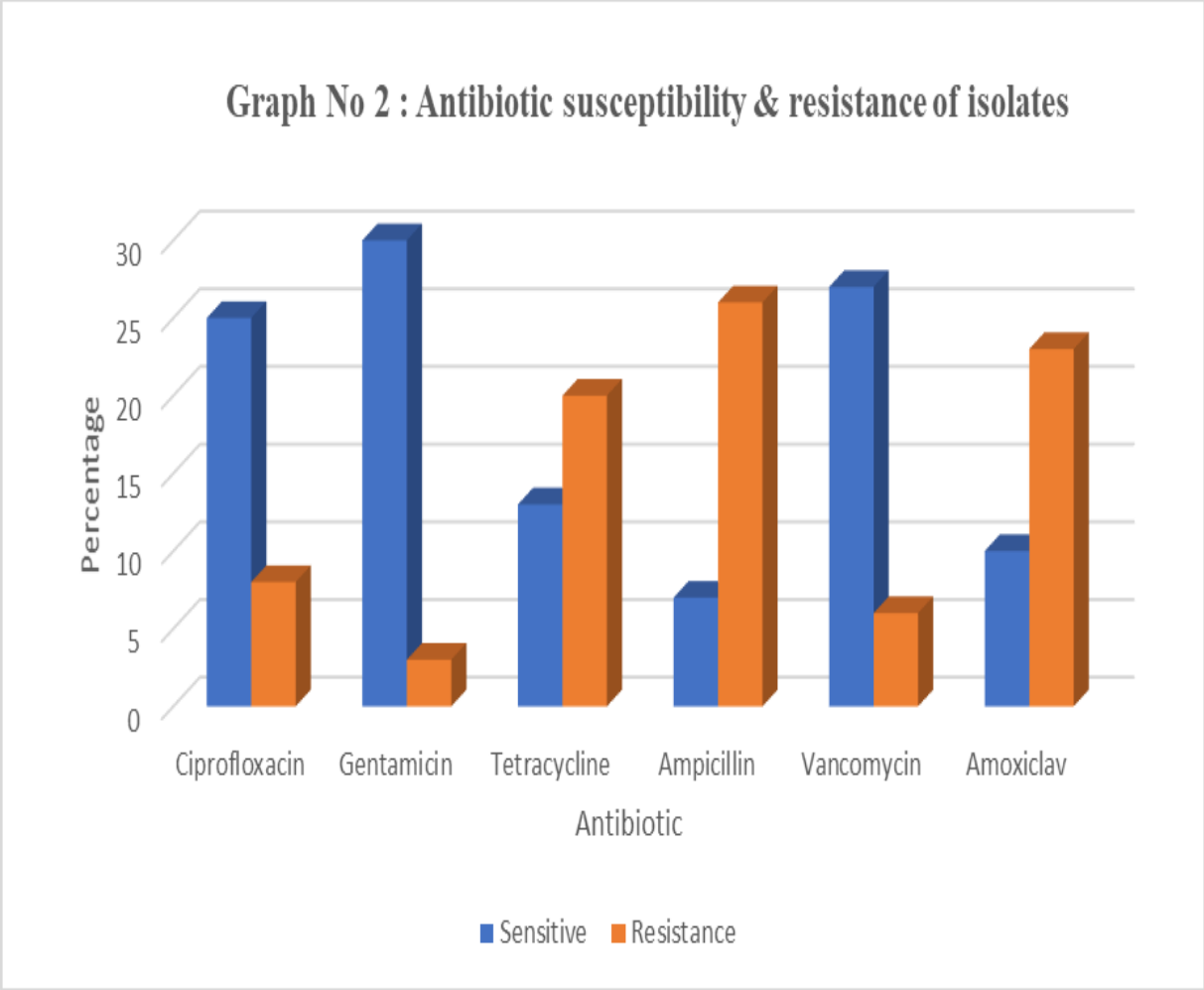
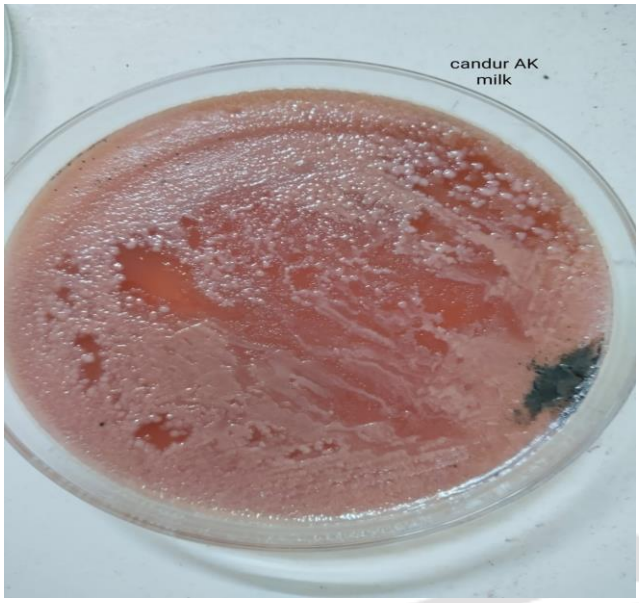


Table 4: Morphological, cultural & biochemical characteristics of isolates

Sr. No.	Characteristics	Isolates		
Morphological characteristic				
1.	Gram Character	Gram -ve rod	Gram -ve rod	Gram -ve rod
2.	Motility	Non-motile	Non-motile	Non-motile
Cultural characteristic				
3.	Shape	Irregular	Irregular	Irregular
4.	Size	0.7-1.2 µm	0.7-1.2 µm	0.7-1.2 µm
5.	Colour	Grey/White	Grey/White	Grey/White
6.	Opacity	Opaque	Opaque	Opaque
7.	Margin	Entire	Entire	Entire
8.	Elevation	Flat	Flat	Flat
9.	Surface	Smooth	Smooth	Smooth
Biochemical characteristic				
Sugar Fermentation				
10.	Glucose A, G	+ve, +ve	+ve, +ve	+ve, +ve
11.	Sucrose A, G	-ve, -ve	+ve, -ve	+ve, -ve
12.	Lactose A, G	-ve, -ve	-ve, -ve	-ve, -ve
13.	Maltose A, G	-ve, -ve	+ve, -ve	+ve, -ve
IMViC Test				
14.	Indole	+ve	+ve	+ve
15.	Methyl Red	+ve	+ve	+ve
16.	Voges Proskauer	-ve	-ve	-ve
17.	Citrate	-ve	-ve	-ve
Enzyme Study				
18.	Catalase	+ve	+ve	+ve
19.	Oxidase	-ve	-ve	-ve
21.	Urease	-ve	-ve	-ve
22.	Gelatinase	+ve	+ve	+ve
23.	Coagulase	-ve	-ve	-ve
24.	H2S Production	-ve	-ve	-ve
Probable Isolates		*Shigella dysenteriae*	*Shigella boydii*	*Shigella sonnei*


Graph No 2 : Antibiotic susceptibility & resistance of isolates
Percentage
30
25
20
15
10
5
0
Ciprofloxacin
Gentamicin
Tetracycline
Ampicillin
Vancomycin
Amoxiclav
Antibiotic
Sensitive
Resistance



Conclusion-

The overall high prevalence of 82.5% (33 out of 40 samples testing positive) indicates widespread Shigella contamination in the region, with significant risks associated with both food and water sources.

Milk and peda showed the highest contamination rates (100% and 90%, respectively), which raises critical concerns regarding hygiene practices in local dairy and sweet production.

Rasgulla and drinking water also displayed notable contamination levels (70%), further underscoring the need for improved sanitation in both food handling and water distribution systems.

In conclusion, this study underscores the urgent need for public health interventions to address the contamination of food and water sources in Akola. Strategies should focus on improving hygiene standards in food production, implementing water purification systems, and strengthening antibiotic resistance monitoring to mitigate the health risks posed by Shigella species.

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