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Antibiotic Susceptibility of Bacterial Isolates from the Sprouts of Mung Bean (*Vigna Radiate* L.)

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Abstract: Mung Bean Sprouts are well known for their high nutritive value and digestibility. They are rich in enzymes, bioavailable vitamins, minerals, amino acids and fibers. Survey of sprouted seeds available at retail vendors has shown the presence of pathogenic bacteria like *E. coli* O157, *Salmonella* and *Listeria monocytogens*, which is of concern for health conscious public. In the present study antimicrobial susceptibility testing of bacterial isolates from mung bean sprouts is done in order to determine which antimicrobial agent is to use against specific strains of bacteria. Antibiotic susceptibility of bacterial isolates from mung bean sprouts was examined against 10 antibiotics viz. Ampicillin, Amoxicillin, Ciprofloxacin, Chloramphenicol, Oxacillin, Gentamicin, Voriconazole, Rifampicin, Methicillin, Norfloxacin.

Introduction

Sprouting is the practice of germinating seeds to be eaten either raw or cooked. Sprouts are believed to be highly nutritious and rich in enzymes which promote good health. Sprouts, including mung beans and alfalfa sprouts, have become a common food item in grocery stores, salad bars around the world.^{1,2,3} A large number of food borne disease outbreaks reported world over have been found to be linked to sprouts. In most instances, the illnesses were caused by either *Escherichia coli* O157:H7 or *Salmonella* bacteria. Anyone who eats raw sprouts or lightly cooked mung bean sprouts is at risk for exposure to *Escherichia coli* O157:H7 or *Salmonella* bacteria.⁴ Thus it is necessary to control the contamination of seeds meant for consumption as sprouts or restrict the growth of pathogens during sprouting. The present study was undertaken to determine the antimicrobial susceptibility of isolated bacteria from mung bean sprouts.

Material and Methods

The present study was carried out in Department of Microbiology, Sardar Bhagwan Singh Post Graduate Institute of Biomedical Sciences and Research, Balawala, Dehradun.

Collection of Sample

Two seed samples of mung bean were purchased from local market from two different retail shops.

Seed Sprouting

Each type of seeds (5.0g) were placed in a sterile petri plate containing Whatmann No. 113 (qualitative wet strength) filter paper saturated with sterilized water or tap water. The seeds were incubated at room temperature (25°C).

Microbial Analysis of Seed Sprouts

Sprouts after 24, 48, 72, 96 h of sprouting from 5.0 g of seeds were suspended in 95 ml sterile water blank. The flasks were placed on a rotary shaker for 15 minute and ten fold serial dilutions were prepared. Aliquots of 0.1 ml of appropriate dilutions were spread on plate containing Mac Conkey Agar, Brilliant Green Agar, Baird Parker's agar and plates were incubated at 37°C for 24 to 48 hrs.

The plates were observed after incubation for growth and colony Characteristics. Number of different colonies appearing on the plates was counted and number of bacteria present on each sprout was calculated as follows

$$\text{No. of Bacteria per Sprout} = \frac{\text{Number of colonies} \times \text{dilution factor} \times 0.1}{\text{Number of seeds}}$$

Cultural Characterization

The colonies were observed for their color, texture, outline, opacity, pigmentation etc and different type of colonies appearing on all the three media were counted.

Morphological Characterization

The colonies were picked and processed for Gram staining technique to differentiate between Gram positive and Gram negative bacteria, shape and arrangement of cells.

Media Composition

Different types of media used in the study were Mac Conkey Agar, Brilliant Green Agar, Baired Parker's agar etc., which were sterilized by autoclaving at 121°C for 20 minute at 15 lbs psi.

Mac Conkey Agar Medium (pH- 7.4) ⁵

Ingredients	Amount (g/ l)
Peptone	: 5.0
Lactose	: 10.0
Bile salt	: 5.0
Sodium chloride	: 5.0
Neutral red	: 0.075
Agar	: 15.0

Brilliant Green Agar Medium (pH- 6.9) ⁶

Ingredients	Amount (g/l)
Lactose	: 10.0
Sucrose	: 10.0
Peptone	: 5.0
Sodium chloride	: 5.0
Phenol red	: 0.08
Brilliant green	: 0.0125
Agar	: 20.0

Baired Parker's Medium (pH-7.0) ⁷

Ingredients	Amount (g/ l)
Casein enzyme hydrolysate	: 10.0
Beef extract	: 5.0
Yeast extract	: 1.0
Glycine	: 12.0
Sodium pyruvate	: 10.0
Lithium chloride	: 5.0
Agar	: 20.0

Muller Hinton Agar (pH 7.3)

Ingredients	Amount (g/ l)
Caesin acid hydrolysis	: 17.5
Beef heart infusion	: 2.0
Starch soluble	: 1.5
Agar	: 18.0

Drug Susceptibility (Intrinsic Antibiotic Resistance) Test

Due to emergence of many antibiotic resistant strains of bacteria, antimicrobial susceptibility testing is done in order to determine which antimicrobial agent to use against specific strain of bacteria. Antibiotic susceptibility was determined by disk diffusion method according to standard microbial procedures. In this method, a standard suspension of bacteria (0.5 turbidity Mc farland standard) to be tested was inoculated on the surface of Muller

Hinton Agar plates. Filter paper disc containing specific concentration of antimicrobial agents were pressed onto the surface and incubated at 37°C over night. After incubation, the zone of inhibition of growth of bacteria around each disc was measured and the susceptibility was determined.⁸ Disc of following antibacterial chemotherapeutic agents were used in this study: Ampicillin (10mcg/disc), Amoxycillin (30/15mcg/disc), Ciprofloxacin (10mcg/disc), Chloramphenicol (10mcg/disc), Oxacillin (1mcg/disc), Gentamicin (120mcg/disc), Voriconazole (1mcg/disc), Rifampicin (15mcg/disc) Methicillin (30 mcg/disc), Norfloxacin (10 mcg/disc).

Result and Discussion

In the present study antibiotic susceptibility testing of bacterial isolates from mung bean sprouts was done. The observations of the experiments conducted were discussed as below:

Morphological Characteristics of Selected Isolates

List of isolates from different samples of mung bean sprouts picked at different time interval is given in table 1.

Table 1: List of isolates from mung bean sprouts

Sample no.	Media	Colony characteristics	Code no.
A	Mac Conkey Agar	Dark pink, flat ,small ,circular	I –A1 ,I-A2 ,I-A3 ,I-A4
		Light pink with centred, gummy, dome shaped, circular	II –A1 ,II-A2 ,II-A3 ,II-A4
	Brilliant Green Agar	Cream, small, circular raised	III –A1 ,III-A2 ,III-A3, III-A4
		Yellow, gummy, large Dome shaped, circular	IV –A1 ,IV-A2 ,IV-A3, IV-A4
	Baired Parker's Agar	Dark black, small, circular	V –A1 ,V-A2 ,V -A3, V -A4
	B	Mac Conkey Agar	Dark pink, flat, small, circular
Light pink with centred, gummy, dome shaped, circular			II –B1 ,II-B2, II-B3, II-B4
Brilliant Green Agar		Cream, small, circular raised	III –B1 ,III-B2, III-B3, III-B4
		Yellow, gummy, large Dome shaped, circular	IV –B1 ,IV-B2, IV-B3, IV-B4
Baired Parker's Agar		Dark black, small, circular	V –B1 ,V-B2, V-B3, V-B4

Drug Susceptibility (Intrinsic Antibiotic Resistance) of Selected Isolates

Antibiotic susceptibility of all the isolates from mung bean sprouts was examined against 10 antibiotics viz Ampicillin (10mcg/disc), Amoxycillin (30/15mcg/disc), Ciprofloxacin (10mcg/disc), Chloramphenicol (10mcg/disc), Oxacillin (1mcg/disc), Gentamicin (120mcg/disc), Voriconazole (1mcg/disc), Rifampicin (15mcg/disc) Methicillin (30 mcg/disc), Norfloxacin (10 mcg/disc). Antibiotic sensitivity pattern of *Escherichia coli* isolated from mung bean sprouts against 10 antibiotics were observed (Table 2). All the 5 isolates were found to be resistant to three antibiotics i.e Voriconazole, Methicillin and Ampicillin. All other isolates were highly sensitive to the remaining 5 antibiotics except isolate I-A3 which was found to be moderately sensitive to Chloramphenicol (zone of inhibition 11mm).

Antibiotic sensitivity pattern of *Klebsiella sp.* isolated from different sprouts is given in table 3. All these isolates were resistance to antibiotics like Voriconazole, Oxacillin, Amoxycillin. One isolate i.e. II-A1 was also resistant to Rifamycin. All the isolates were highly sensitive to Chloramphenicol, Gentamycin, Voriconazol, Rifamycin and Norfloxacin. Antibiotic sensitivity of 4 isolates of *Salmonella sp.* and 2 isolates of *Shigella sp.* isolated from mung bean sprouts on Brillant Green agar against 7 antibiotics is given in table 4 and 5. On the basis of size of zone of inhibition all the isolates of *Salmonella sp.* and *Shigella sp.* were found to be resistant to the Voriconazole and Oxacillin. Two isolates viz III-A4, III-B4, were moderately sensitive to Amoxicillin. Antibiotic sensitivity pattern of *Staphylococcus aureus* isolated from mung bean sprouts was also studied. The observations are given in table 6. Eight isolates were tested and found to be reistant to Voriconzole, Methicillin and Oxacillin and were highly sensitive to the 5 remaining antibiotics namely Amoxycillin, Chloromphenicol, Amipicillin, Gentamycin and Ciprofloxacin.

Table 2: Antibiotic Sensitivity Pattern of *E. coli* isolated from Mung Bean Sprouts

Isolate no.	Antibiotics (Zone of Inhibition mm)							
	Cf ¹⁰	C ¹⁰	AS ¹⁰	G ¹²⁰	VOR ¹	Ox ¹	Rf ¹⁵	M ³⁰
I-A3	32	11	–	25	–	26	13	–
I-A4	30	26	–	19	–	28	19	–
I-B2	25	24	–	21	–	32	18	–
I-B3	22	19	–	20	–	22	17	–
I-B4	24	21	–	22	–	24	11	–

G – Gentamicin , VOR –Voriconazole , C-Chloromphenicol, Ox- Oxacillin,
 Cf - Ciprofloxacin, Rf -Rifampicin , A –Ampicillin, M-methicillin
 Figures in superscript indicate concentration (mcg) of antibiotics.

Table 3: Antibiotic Sensitivity Pattern of *Klebsiella* isolated from Mung Bean Sprouts

Isolate no.	Antibiotics (Zone of Inhibition mm)							
	Cf ¹⁰	C ¹⁰	AS ¹⁰	G ¹²⁰	VOR ¹	Ox ¹	Rf ¹⁵	Nf ¹⁰
II-A1	21	22	–	19	–	–	9	25
II-A2	20	24	–	25	–	–	13	24
II-A3	25	27	–	30	–	–	15	22
II-A4	29	21	–	24	–	–	19	26
II-B1	34	29	–	19	–	–	11	24
II-B2	29	25	–	21	–	–	11	24
II-B3	26	28	–	19	–	–	17	22
II-B4	21	27	–	19	–	–	14	20

G–Gentamicin, VOR-Voriconazole, C - Chloromphenicol, Cf - Ciprofloxacin
 Rf - Rifampicin , A- Ampicillin, Nf- Norfloxacin, Ox- Oxacillin
 Figures in superscript indicate concentration (mcg) of antibiotics

Table 4: Antibiotic Sensitivity Pattern *Salmonella* spp isolated from Mung Bean Sprouts

Isolate no.	Antibiotics Zone of inhibition (mm)						
	CF ¹⁰	C ¹⁰	AS ¹⁰	G ¹²⁰	VOR ¹	OX ¹	AMS ^{30/15}
III-A1	33	26	26	29	–	–	16
III-A4	30	22	19	22	–	–	14
III-B4	31	21	24	26	–	–	12
III-B3	32	23	23	29	–	–	19

G–Gentamicin ,VOR –Voriconazole , C-Chloromphenicol , Cf - Ciprofloxacin
 A -Ampicillin, OX – Oxacillin, AMS –Amoxacillin, M- Methicillin
 Figures in superscript indicate concentration (µg) of antibiotics

Table 5: Antibiotic Sensitivity Pattern *Shigella* isolated from Mung Bean Sprouts

Isolate no.	Antibiotics Zone of inhibition (mm)							
	CF ¹⁰	C ¹⁰	AS ¹⁰	G ¹²⁰	VOR ¹	OX ¹	AMS ^{30/15}	M ³⁰
III-A2	27	21	18	26	–	–	13	–
III-B1	27	25	25	24	–	–	16	–

G – Gentamicin , VOR –Voriconazole , C-Chloromphenicol , Cf - Ciprofloxacin
 A - Ampicillin, OX – Oxacillin, AMS –Amoxacillin, M- Methicillin
 Figures in superscript indicate concentration (µg) of antibiotics

Table 6: Antibiotic Sensitivity Pattern of *Staphylococcus aureus* isolated from Mung Bean Sprouts

Isolate no.	Antibiotics						
	Zone of inhibition (mm)						
	Cf ¹⁰	C ¹⁰	AS ¹⁰	G ¹²⁰	VOR ¹	OX ¹	AMS ^{30/15}
V-A1	30	20	21	26	–	–	28
V-A2	32	22	26	24	–	–	29
V-A3	31	27	26	29	–	–	27
V-A4	29	22	28	26	–	–	29
V-B1	32	27	25	25	–	–	30
V-B2	32	27	24	27	–	–	31
V-B3	27	25	29	29	–	–	33
V-B4	34	19	30	28	–	–	28

G–Gentamicin, VOR–Voriconazole , C- Chloromphenicol , Cf - Ciprofloxacin, AS-Ampicillin, OX–Oxacillin, AMS –Amoxycillin

Figures in superscript indicate concentration (mcg) of antibiotics

Summery and Conclusion

The present study was undertaken to determine the antimicrobial susceptibility of isolated bacteria from mung bean sprouts against 10 antibiotics viz. Ampicillin, Amoxycillin, Ciprofloxacin, Chloramphenicol, Oxacillin, Gentamicin, Voriconazole, Rifampicin, Methicillin, Norfloxacin.

- The isolates were picked from different samples on two types of media and purified for morphological characterization. They were found to belong to *Escherichia coli*, *Klebsiella*, *Salmonella*, *Shigella* and *Staphylococcus aureus*.
- The antibiotic susceptibility pattern of all the isolates was also determined. Isolates found from *Escherichia coli* were resistant to Ampicillin, Methicillin, and Voriconazole whereas isolates belonging to *Klebsiella spp.* were resistant to Ampicillin, Voriconazole, and Oxacillin.
- All the isolates of *Salmonella spp.* and *Shigella spp.* isolated on Brilliant Green Agar were resistant to Oxacillin and Voriconazole. Likewise all the isolates of *Staphylococcus aureus* isolated on Baird Parker's medium were also resistant to Oxacillin, Methicillin and Voriconazole.
- Thus, care should be taken in the consumption of the raw sprouts irrespective of their source i.e. whether prepared at home or procured from the retail market.

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