Indian J. Vet. Med. Vol. 42, No. 1, 2022 pp. 28-32

Evaluation of antibacterial activity of ayurvedic nanoparticle drug *Rasamanikya* against the ESBL producing *E. coli*

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Abstract

Day-by-day there is an increase new resistant strains bacteria so as to counteract it various nanomaterial drugs are being studied for testing their efficacy. In the present experiment *Rasamanikya,* a reported ayurvedic herbo-metallic nanoparticle drug has been used to evaluate the antimicrobial activity against ESBL producing *E. coli* strains that exhibited resistance to three or more clinically important veterinary antimicrobials and were of diverse food-animal origin. In order to investigate the antibacterial effects of the *Rasamanikya* (RM), we employed *in-vitro* tests – agar well diffusion method, plate diffusion method and disc diffusion method.

Keywords: MDR, Nanoparticle, Rasamanikya, in-vitro tests.

We know that day-by-day there is an increase in new resistant strains of bacteria due to use of huge amount of antibiotics in human as well as animal therapy resulting in development of resistance to antimicrobial drugs which leads to ineffective treatment, persistence and spread of infections. Although MDR development is a natural phenomena and an increasing worldwide public health concern, it is urgent to find out new and effective alternative for combating such multi-drug resistant isolates (Poole 2004, Michelle *et al*., 2010) that's why various nanomaterial drugs or nanomedicines are being studied for testing their efficacy (Singh *et al*., 2008). Nanomedicines are nanoparticles based drug formulations containing ultra-fined particles generally ranges from 1-100 nm in diameter (Vert *et al*., 2012). Many nanoparticle based drugs show potential antibacterial activity against various Gram +ve and Gram -ve bacteria including highly methicillin and carbapenem resistant strains and increasing their exposure among animals and human (Nazifi *et al*., 2015, Stebounova *et al*., 2011). In the present era, nanotechnology is a rapid growing technology which plays an important role on various fields of therapeutic applications and capable of solving several problems related to animal health and production (Youssef *et al*., 2019) with the principle to prevent the adverse effects of loaded drugs by reducing the amount of drugs. Ayurvedic system of medicine is one of the oldest systems of Indian traditional medicine and various medicines are based on metal-mineral formulation commonly known as Bhashma (Farooq *et al*., 2018). In the herbo-metalic preparation, the particle that are being used, have a diameter of about 10-15nm. The renovated review on scientific literature on types of Bhashma and their therapeutic efficacy along with metal nanoparticles has been observed on various types of Bhashma. The combination of ayurveda and nanotechnology may provide the best solution as a medicine to treat various life-threatening diseases (Kaur *et al*., 2018). Mineral rich Rasamanikya Nanoparticle (RMNP) which is ayurvedic herbo-metallic nanomedicine having antimicrobial and anticancer potential assessed by in-vitro cellular assay (Ruidas *et al*., 2019), has been commonly used in various skin diseases, bronchial asthama, eczema, fistula, gout and syphillis (Acharya 2001). In the present study RMNP is used for evaluation of antimicrobial activity against ESBL producing *E. coli* strains.

Materials and Methods

A. Preparation of drug Rasamanikya

Raw Haritala (orpiment) was purchased from local market in Kolkata, India. The sample was identified and authenticated by the scientist in the J. B. Roy State Ayurvedic Medical College & Hospital, Kolkata, India. The voucher sample (RM/11/19) was submitted in the Pharmacy Department.

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Raw orpiment was made into small pieces. The pieces were taken in a cloth parcel. The parcel was put in a vessel and was boiled in lime water for three hours. The orpiment pieces were then collected, dried and made into coarse powder (22#) form. The powder was taken in between two mica leaves; and was heated at 400^{O}C temperature to melt. After complete melting, the arrangement was taken off from heating device. It became solid after cooling down. The material was collected from the mica leaves and was made into powder form. The powder was stored in a glass bottle and was used for the experimental study.

B. Antimicrobial sensitivity test for Rasamanikya

The in-vitro experiment for assessment of antimicrobial sensitivity profile against MDR bacterial strains of E. coli was performed in the laboratory of Indian Veterinary Research Institute (IVRI), Kolkata in West Bengal state under the supervision of one of the authors. We employed agar well diffusion method, plate diffusion method and disc diffusion method. In total 63 *E. coli* strains that exhibited resistance to three or more clinically important veterinary antimicrobial and were diverse food-animal origin were included for screening.

I) Agarwell diffusion method

Muller-Hinton Agar medium was prepared, sterilized and poured into the sterile petriplates and was allowed to solidify. By using cotton swab Culture of *E. coli* strain was uniformly spread on to the plates containing media. 1 mg of Rasamanikya was mixed with 1 ml of Solvent (DMSO/ Diethyl ether/ Chloroform/ Distilled water) to prepare a suspension. By using gel puncture well was made in the agar media and the well was filled with the suspension. Each hole contain 200 µl of suspension. The plates were kept carefully in incubator for overnight.

II) Plate diffusion method

250 ml Muller-Hinton Agar media is mixed with different concentration of Rasamanikya i.e. 2.5 mg, 5 mg and 10 mg.The media was placed into sterilized petriplates and leave it to solidify. By using markar pen, boxes were drawn for placement of Bacterial culture (different strains *of E. Coli)* in each box.1 µl of 0.5 MacFarlane Bacterial culture were placed into each boxes. After drying the petriplates were kept in incubator for overnight.

III) Disc diffusion method

Muller-Hinton Agar medium was prepared,

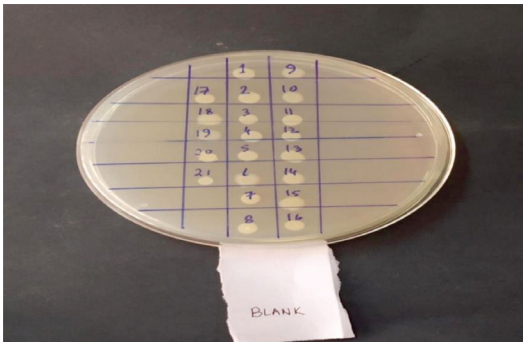

Fig. 1. Plate diffusion method without drug

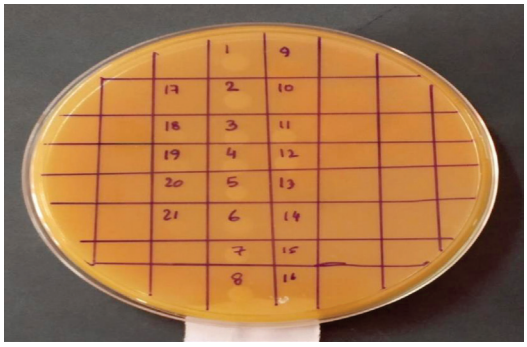

Fig. 2. Plate diffusion method at 5 mg concentration

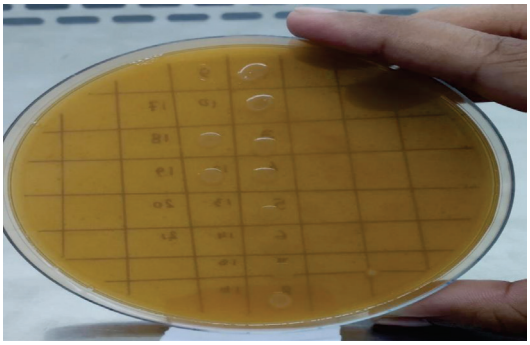

Fig. 3. Plate diffusion method at 10 mg concentration

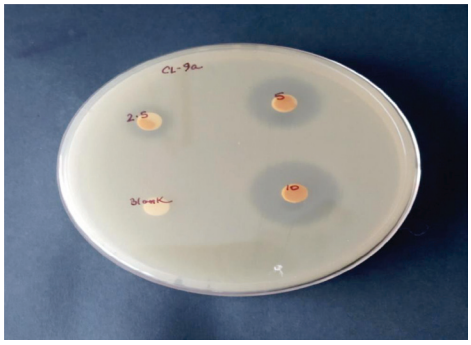

Fig. 4. Disc diffusion method showing zone of inhibition

SL. No.	Isolates name	Blank (mm)	2.5 mg/ml (mm)	5 mg/ml (mm)	10 mg/ml (mm)
1.	CL9a	6	11	18	21
2.	SL14a	6	6	17	21
3.	SL11a	6	17	19	23
	SL11b	6	15	14	23
4.	5923a	6	15	23	24
	5923b	6	11	19	24
5.	5929a	6	13	21	22
	5929b	6	20	25	31
6.	15121/b	6	11	10	24

sterilized and poured into the sterile petriplates and was allowed to solidify. By using cotton swab culture of *E. Coli* strain was uniformly spread on to the plates containing the media. To make suspension 100 mg of Rasamanikya was dissolved in 250 µl of Methanol and 500 µl of distilled water. By using Micropipette measured volume of suspension were taken in each disc to make Different concentration i.e. 2.5 mg, 5 mg, 10 mg for evaluation of zone of inhibition. After drying the discs were place on the previously swabed petriplates.The plates were kept in incubator for overnight.

Results and Discussion

In order to investigate the antibacterial effects of the Rasamanikaya (RM), we employed *in-vitro* tests – agar well diffusion method, plate diffusion method and disc diffusion method. In total, 63 *E. coli* strains that exhibited resistance to three or more clinically important veterinary antimicrobials and were of diverse food-animal origin were included for screening. In agar well diffusion method, the isolates, in triplicate, were tested to increasing concentration of RM in MH agar (0-10 mg). Disc diffusion test was conducted in MH agar using the paper discs impregnated with different concentrations of RM – 0 mg, 2.5 mg, 5 mg and 10 mg. In agar well diffusion method, none of the extract of Rasamanikya showed the antimicrobial activity against these 63 strains of *E. coli* and out of them only 6 strains were able to inhibit the bacterial colony growth on 5 mg and 10 mg concentration of Rasamanikya in plate diffusion method (Fig 1, 2, 5 and 6) depicted in Table 1. These 6 strains were taken for further study to evaluate the zone of inhibition (ZOI). Reduction in the number of colonies of the test isolates with exposure to the drug was considered as an evidence of their antibacterial effect in plate diffusion method. Likewise, in disc diffusion test, increase in the inhibition zone around the impregnated discs was considered to appreciate the antibacterial effect. However, RM in 5 and 10 mg discs caused in significant increase in the zone of inhibition in six ESBL producing isolates (Fig 4 and 7). The details of the result are illustrated in Table. 2. It appears from the study that RM has some antibacterial effect against the ESBL producing *E. coli* of animal origin, though it effect was only evident in disc diffusion test. In agar dilution test, we have tested RM with several diluents - distilled water, DMSO, ethyl alcohol, chloroform and diethyl ether, but none was able to mirror the results of disk diffusion test. Solubility and diffusion of drugs are two important factors to determine the effectiveness of the drug molecules in agar dilution test. It is not clear if incompatibility of the drug molecules with MH agar failed to produce the results obtained in disk diffusion test. Further, drugs are usually added to the media in its liquid state to ensure uniform distribution of the drug around 50 to 55^0C. It is not yet known if this temperature range has any effect on stability and activity on RM. Rasamanikya is considered a magic drug by Ayurvedic physicians and it has long been used for Vata-Kaphaja diseases like Shwasa, Kasa and Kushtha (Skin disorders). At the same time, the drug earned controversy because of the presence of arsenic and sulphide – compounds that can cause hazardous health effects among the consumers. Nevertheless, Ayurveda refuted such possibility claiming their preparation having very small concentration of such compounds, that too in purified forms. However, a recent study showed that RM induced mild to moderate fatty changes in liver (Ayu. 2013 Jul;34(3):309-15. doi: 10.4103/0974-8520.123134). Therefore, the safety concern of the drug remains unresolved. Till date, only little literature is available to support the antibacterial effect of RM (Ruidas *et al*.,

Table 1: Results of plate diffusion method for RM

Diffusion method	Blank (mm)	2.5 mg/ml (mm)	5 mg/ml (mm)	10 mg/ml (mm)
Positive	63(100%)	63(100%)	57(90%)	57(90%)
Negative	0 0 (0%)	0 0 (0%)	06 (10%)	06 (10%)

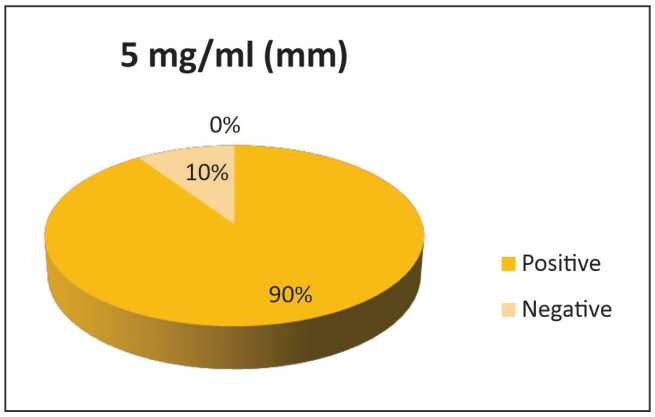

Fig. 5

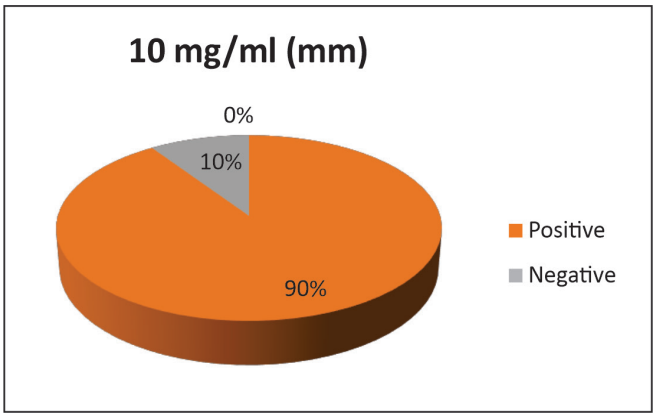

Fig. 6

Table 2: Results of Disc Diffusion method for RM

diffusion method	Blank (mm)	2.5 mg/ml (mm)	5 mg/ml (mm)	10 mg/ml (mm)	P-value
Isolates	6±.00^{d}	13.22±1.37^{c}	18.44±1.52^{b}	23.67±1.00^{a}	<.001**

Table value represent Mean± Standard Error
** Significant at 1% level of significance
Different superscripts (a,b,c and d) differ significantly at 1% (P<.01) level of significance according to Tukey's HSD test.

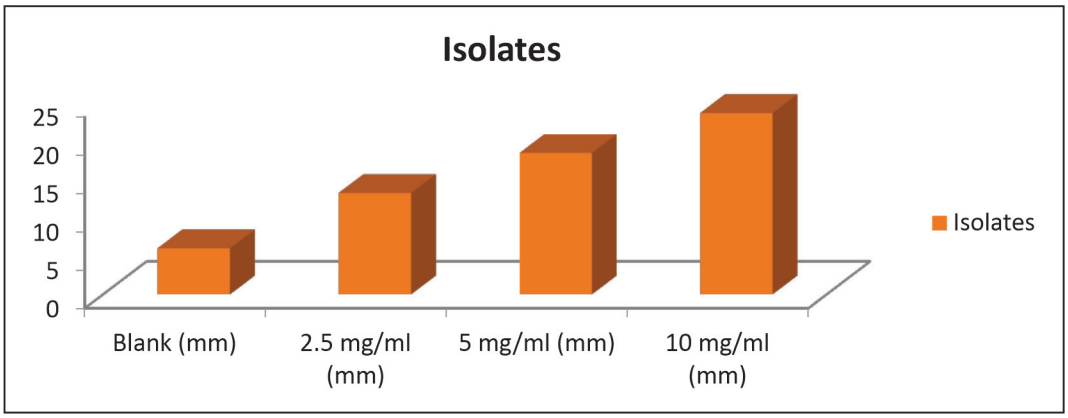

Fig. 7

2019). However, their use in Kushtha (leprosy) gives an indication that the drug might be effective in other bacterial diseases, as well. RM contains arsenic – a heavy metal known to have antibacterial effect. In fact, a new natural arsenic containing antibiotic, arsinothricin or AST has been recently discovered having potential broad spectrum antibacterial effect (Venkadesh *et al*., 2019). It remains to see whether RM mediates its antibacterial effect in the similar manner.

Conclusions

It has been concluded that ayurvedic herbo-metallic nanoparticle drug *Rasa Manikya* has antibacterial effect against the ESBL producing *E. coli* of

animal origin in plate diffusion method and disc diffusion test, whereas no effect was found in agar dilution test.

Acknowledgement

The author would like to acknowledge Dr. Prasanta Kumar Sarkar, Professor and H.O.D, Department of Rasashastra, J.B. Roy State Ayurvedic Medical College and Hospital, Kolkata (W.B.) for providing the test drug *Rasamanikya*, Dr. Samiran Bandapadhyay, Senior scientist, Indian Veterinary Research Institute (IVRI), Kolkata to allow in the institute laboratory for the experiment under his supervision and All lab technicians for their co-operation during whole experiment.

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Received : 25.02.2022
Accepted : 27.05.2022