

# Study of antimicrobial activity of two common anti-cough formulations sold in Bangladesh

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**Coughing is a physiological process of clarifying the mucosal obstruction through the airways is often be complicated by the invasion following infection by an array of microorganisms. A number of anti-cough formulations are available to combat coughing which is a non-antibiotic drug by nature; however, in some instances these drugs may exhibit anti-microbial trait in action probably due to the chemical properties of the contents. Present study aimed to determine such anti-microbial activity of two commonly used anti-cough mixtures which are locally formulated by the reputed pharmaceuticals in Bangladesh. The antibiotic susceptibility pattern of five laboratory strains of *Escherichia coli*, *Klebsiella sp.*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were tested by the agar well diffusion method against these two oral liquid samples under investigation used as anti-cough drugs. Sample 1 was found to be highly effective against all of the five test organisms whereas sample 2 was found to be effective against four of the tested organisms. Interestingly, even the lowest amount (50 µl) of both drugs were able to mount inhibitory action against at least two test organisms, the highest amount of both drugs have been found to pose more antimicrobial action than their positive control Gentamycin 10 mg.**

**Key words:** Drugs; Oral liquids; Coughing; Anti-microbial activity; Microorganisms

Cough is a suddenly occurred repetitive reflex with characteristic sound that expulse air from the lungs to clear obstructions like -secretions, irritants, foreign bodies and microbes from the respiratory tract (1, 2). It is one of the most common complaints persuading patient visits to healthcare professionals and the second most common reason for a general medical diagnosis (2, 3). Cough is not a disease, instead it is the symptom of disease that occurs in respiratory tract (4) which can indicate serious illness if occurs too frequently. Most of the time, a respiratory tract infection by bacteria or virus cause irregular coughing (2) but it can also be triggered by choking, air pollution, smoking (1) and other medical conditions. As a common household disease many patients take prescribed or non-prescribed medicaments to treat coughing (5, 6).

Antitussive or cough suppressant and expectorant are the two basic short acting syrups for cough management. Suppressants like dextromethorphan, temporarily reduce coughing to normal level when its intensity is excessive (7, 8) and are generally considered as safe at recommended doses. Expectorant attempts to dampen excessive mucus production by liquefaction which improves the removal of secretions by ciliary

transport and/or by coughing (8-10).

In Bangladesh, Pharmaceutical manufacturers use Dextromethorphan hydrobromide, Pseudoephedrine hydrochloride, Triprolidine hydrochloride, Bromohexin hydrochloride etc. according to the guideline of USP and BP in both adult and children's cough mixtures. Often found in liquid formulation, these cough medicines sometimes contain antibacterial activity which is not commonly indicated in the remedy. Again, a common problem with liquid formulation is difficulty of precise measurement. Therefore, taking low or excessive dose than that of recommendation can result in drug resistance among the microorganisms of respiratory tracts and other body parts. Study of Antimicrobial activity of common cough mixtures is inadequate or has not been done yet. Hence, this study will examine the antimicrobial effect of antitussive and expectorant syrup commonly available and used in Bangladesh.

## MATERIALS AND METHODS

**Study site, sample collection and sample processing.** Samples were randomly collected from drug store during August, 2016, according to the standard sampling method suggested by American Public Health Association (11). During collection both drug satisfied the manufacturing and expiry dates and storage criteria.

**Determination of antimicrobial activity by agar well diffusion method.** To observe in vitro antimicrobial activity, a standard procedure of agar well diffusion method was performed (12, 13). Agar well diffusion assay was observed against five common opportunistic pathogen found in respiratory tract. *Escherichia coli*, *Klebsiella sp.*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus cereus* were the organisms tested in this study. Broth cultures of these organisms in Muller-Hinton Broth (MHB) were adjusted to the turbidity of Macfarland

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standard of 0.5 at first. Then with sterilized cotton swab, bacterial suspension was evenly spread on Muller-Hinton agar for confluent growth of each organism. With sterile cork-borer, four well of different size were made on agar plate to assay antimicrobial action. According to the size of the well different amount of drug 50  $\mu$ l, 150  $\mu$ l, 200  $\mu$ l and finally 500  $\mu$ l was added in the wells. For both positive and negative controls, disk of gentamicin 10  $\mu$ g and sterile MHB were used respectively. After overnight incubation at 37 °C, presence of clear zone was indicative of the active compound in the tested samples against the five pathogens (14, 15)

## RESULTS AND DISCUSSIONS

Antimicrobial activities of non-antibiotic compounds have been demonstrated in several studies (2, 16, 17). In another study in Nigeria antimicrobial activity of cough syrups was observed as well (2). But no data was found on similar activity on locally produced cough mixtures, although these over the counter drugs are very commonly used. Our current study was aimed to investigate the antimicrobial effect of locally produced oral cough syrups. Sample 1; being adult's cough suppressant, contains Dextromethorphan hydrobromide (DXM) BP 10 mg, Pseudoephedrine hydrochloride (PPH) BP 30 mg and Triprolidine hydrochloride (TRL) BP 1.25 mg in 5 ml of mixture. And sample 2, children's expectorant contains 4 mg of Bromhexin hydrochloride (BXH) in 5 ml formulation.

Pharmaceutical form rather than pure form of these two formulations were analyzed against five common pathogens. Both of the medicine showed significant amount of inhibitory effect as sample 1 was found inhibitory against all of the five test organisms and sample 2, inhibited growth of all organisms except *Pseudomonas aeruginosa*. When compared with the results of inhibition with the positive control Gentamycin 10mg, inhibiting activity was greater with the test drugs. Result of antimicrobial activity by agar

well diffusion is included in table 1.

Against *E.coli* both sample 1 and sample 2 showed effective killing and diameter of clear zone was proportional with increased amount of drug concentration. Sample 1 mounted clear zone with diameter ranging from 18 to 26 mm with 50 to 500  $\mu$ l, whereas, sample 2 produced clear zone with diameter ranging with 18 to 30 mm with similar amount of cough mixtures. Gentamycin 10 mg showed a much smaller clear zone and the diameter was found only 12 mm.

For *Klebsiella* spp. 50  $\mu$ l of both sample drugs failed to clear bacterial growth. Sample 1 found effective at 200  $\mu$ l. Diameter of clear zone was 13 mm and 26 mm for 200  $\mu$ l and 500  $\mu$ l respectively. Sample 2 could mount killing effect at 150  $\mu$ l compared to 200  $\mu$ l of sample 1 and diameter of inhibition area ranged from 15 to 21 mm. Again Gentamycin was much less effective, producing only 10 mm diameter of clear zone.

Only one pathogen *Pseudomonas aeruginosa* remained unaffected or resistant against sample 2, expectorant syrup. No inhibition of growth was observed even with the highest concentrations of this drug. But for sample 1, growth inhibition showed a similar pattern that with *E.coli* resulting in clear zone with diameter 11 mm with both 50 and 150  $\mu$ l. 12 mm and 25 mm were found with 200  $\mu$ l and 500  $\mu$ l respectively. For Gentamycin clear zone exhibited a diameter of 20 mm.

Both the sample drugs were found to have antimicrobial activity against gram positive bacteria. 50  $\mu$ l of sample 2 could not inhibit the growth of *B. cereus* and *S. aureus*. Interestingly, 150 and 200  $\mu$ l were equally inhibitory as diameters of clear zones were equal, 11 mm for *B. cereus* and 16 mm for *S. aureus*. 25 mm and 30 mm diameter were found for sample 1 against *B. cereus* and *S. aureus*

TABLE 1. Zone of inhibition by agar well diffusion (diameter in mm) against different concentration of cough mixtures

Organism	Zone of inhibition by agar well diffusion (diameter in mm)								
	Sample 1				Sample 2				Gentamycin 10mg  (positive control)
	50 $\mu$ l (.41mg)	150 $\mu$ l (.82mg)	200 $\mu$ l (1.23mg)	500 $\mu$ l (4.1mg)	50 $\mu$ l (.04mg)	150 $\mu$ l (.08mg)	200 $\mu$ l (.12mg)	500 $\mu$ l (.4mg)	
<i>Escherichia coli</i>	18	18	20	26	18	20	22	30	12
<i>Klebsiella spp.</i>	0	0	13	20	0	15	20	21	10
<i>Pseudomonas aeruginosa</i>	11	11	12	25	0	0	0	0	20
<i>Bacillus cereus</i>	0	11	11	25	7	12	16	23	15
<i>Staphylococcus aureus</i>	0	16	16	30	0	0	10	20	10

respectively, showing sample 1 more effective against *S. aureus*.

Sample 2, children's expectorant mixture was found inhibitory with increased concentrations. Growth of *B. cereus* was inhibited progressively. 7 mm, 12 mm, 16 mm and 23mm respectively with 50, 150, 200 and 500 µl. For *S. aureus*, inhibitory activity was lesser than that found against *B. cereus*. 10 and 20 mm against 200 µl and 500 µl. Interestingly Gentamycin was found less inhibitory against both these organisms when compared with sample 1 and sample 2. Clear zone was found with diameter of 15 mm and 10 mm against these two pathogens.

A comparison between the two test samples shows 500 µl of sample 1 produced larger zone of inhibition than that of 500 µl of sample 2. Average diameter of inhibition zone was 25.5 mm for sample 1 compared to 18.8 mm for sample 2. Gentamycin produced an average of 13.4 mm diameter against five pathogenic organisms.

A total of 18 mm diameter of clear zone against *E. coli* and other organisms was also found in previous studies which are supportive to present data (2, 18). Active compounds used in the two studied cough mixtures are mostly non antibiotic with antitussive, decongestant and sometimes with antioxidant activity. But Triprolidine hydrochloride in sample 1 is a known antihistamin compound and antimicrobial activity of some antihistamin compounds have been reported in an experiment (18).

### CONCLUSION

Present study is an indication of antibacterial activity of two commonly used cough mixtures, which were previously known as non-antibiotic. This study shows the cough mixtures were inhibitory to bacterial growth in very little amounts than broad spectrum Gentamycin 10 mg. Further investigation should be conducted to find the underlying reason of this high antimicrobial activity.

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