

# Assessment of pathogenic micro-organisms in commercially available smokeless tobacco products

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To The Editor,

Tobacco is the single most preventable cause of death, and it is well established that they contain harmful chemicals and carcinogens. Pathogenic micro-organisms may be associated with smokeless tobacco products (STPs), which may pose serious public health consequences. Hence, there is a need to assess the presence of pathogenic micro-organisms in these STPs.

Popular brands of gutka, khaini, zarda, unprocessed tobacco and tobacco with lime commercially available with the vendors of Mangalore City, India were used for the study. One gram of the product was transferred into 1 ml of brain heart infusion broth. One microliter of a 1/100 dilution of the original solution was used to inoculate culture media for bacterial and fungal growth. Sheep blood agar and MacConkey's agar were used to isolate bacteria, and Sabouraud dextrose agar was used for fungi. Sheep blood agar and MacConkey's agar was incubated at 37°C for 48 h and Sabouraud dextrose agar was incubated at 37°C for 96 h. Bacterial growth on sheep blood agar and MacConkey's agar were identified by colony morphology, gram staining, motility and biochemical tests. Colony count was done by counting the colonies under the colony counter and multiplying with the dilution factor. Fungal growth was identified based on morphology and tease mount.

*Genus Bacillus* was isolated from khaini, zarda and unprocessed tobacco. *Klebsiella* was isolated from gutka. *Aspergillus fumigatus* was isolated from gutka as shown in Table 1.

The STP products investigated included multiple samples of gutka, khaini, and zarda, and one sample each of unprocessed tobacco and tobacco with lime. Unprocessed tobacco is sun dried tobacco leaves without any additives. Zarda is a commercial mixture in which tobacco leaves are

**Table 1: Micro-organisms isolated from STPs**

STPs	Microorganism isolated		
	<i>Genus Bacillus</i> CFU/g of the product	<i>Klebsiella</i> CFU/g of the product	<i>Aspergillus fumigatus</i>
Gutka-1	-	18×10 <sup>5</sup>	-
Gutka-2	-	19×10 <sup>5</sup>	-
Gutka-3	-	20×10 <sup>5</sup>	-
Gutka-4	-	19×10 <sup>5</sup>	Present
Khaini-1	8×10 <sup>5</sup>	-	-
Khaini-2	15×10 <sup>5</sup>	-	-
Zarda-1	10×10 <sup>5</sup>	-	-
Zarda-2	-	-	-
Unprocessed tobacco	12×10 <sup>5</sup>	-	-
Tobacco with lime	-	-	-

CFU: Colony-forming units, STPs: Smokeless tobacco products

broken up and boiled with lime and spices until dry. Khaini is made up of powdered sun dried tobacco and slaked lime.<sup>[1]</sup> Gutka is a dry preparation of crushed arecanut, slaked lime, catechu, sundried chopped tobacco, saffron and flavoring agents mixed together and sweetened.<sup>[2]</sup> Differences in the manufacturing processes may contribute to the variations in the microorganism species and concentrations (colony counts) found in these products.

The present study detected high colony counts of two potential human bacterial pathogens in several of the samples [Table 1]. The *Genus Bacillus* contains aerobic, gram-positive spore-forming rods. In addition to anthrax, members of the genus cause food-borne infections, gastroenteritis, and a variety of opportunistic infections.<sup>[3]</sup> *Klebsiella* isolated from gutka is known to cause pneumonia, urinary infection and other pyogenic infections like abscesses, meningitis and septicaemia.<sup>[4]</sup> *A. fumigatus* was isolated from one of the four gutka samples. This organism causes fungus balls and allergic pulmonary aspergillosis in the immunologically normal, and can cause invasive infections primarily in debilitated or immune compromised persons. Such infections mainly occur in the lungs but may disseminate to other organs.<sup>[5]</sup> All these infections tend to be more severe in immunocompromised patients.<sup>[3-5]</sup>

More studies are required to identify the presence of pathogenic micro-organisms in a larger numbers of samples of STPs and the effects imposed on its users which should be brought to the notice of the consumers as well as the manufacturers.

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