

## Shift in Oral Commensals During A 5-Day Metronidazole Therapy

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### Abstract

**Background:** Antibiotic treatment induces great ecological impact on normal flora of the human body. Suppression of growth on these commensal microorganisms may give rise to opportunistic infection especially to those who are immuno-compromised. In the mouth, oral thrush represents a common indication of an opportunistic infection by fungus of the genus *Candida*. This study aimed to assess the effect of a 5-day metronidazole therapy on oral microbes to enable evaluation of its influence on the candidal, streptococci and other bacterial inhabitants of the oral cavity.

**Methods:** Tongue scrape specimens from ten patients who were due for minor oral surgery were collected and processed using conventional microbiological procedures involving spreading of specimens on selective agar plates. The specimens were collected on the first, second and a day after the 5-day metronidazole therapy.

**Results:** Distribution of oral candida, streptococci and other bacterial inhabitants experienced changes over the duration of treatment. An increased candidal population from 34.2% before to 45.3% during, and a contrasting decreased in streptococcal presence from 41% before to 28% during the therapy were observed. Similar trend of reduction was obtained on other bacteria from 37% before to 24% over the antibiotic therapy. The influence of metronidazole seem to slowly diminish upon completion of the 5-day therapy as all three microbial groups adapt to regain the original distribution pattern.

**Conclusion:** The course of a 5-day metronidazole treatment affected microbial balance of the oral residents that lead to a significant rise of opportunistic species to take advantage of the disturbed ecological balance.

**Keywords:** Oral candida, Oral streptococci, Growth suppression, Microbial shift.

### Introduction

Antibiotic is often prescribed to patients before and following surgery to suppress the growth and flourish of microorganisms that may interfere with the body recovery process. In addition to compromised immune system which often occur following surgery, the prescription of non-specific antibiotic may eliminate millions of beneficial bacteria that help keep indigenous fungi within ratio that benefit the overall health of the oral cavity and the rest of the body. Health and disease in the mouth are active processes in which the ecology of communities, not of single

organisms, is paramount.

Depending on the type of antibiotic used, its mode of action and the degree of resistance in the microbial community, growth suppression of certain type of commensal species may cause a marked shift in the ecological balance of the mouth. It was reported that even a short-term antibiotic administration can lead to stabilization of resistant bacterial populations in human such as the intestine to persist for years [1, 2]. There are high risks that prolonged antibiotic therapy could lead to increased prevalence of antibiotic resistant microorganisms. Animal models demonstrate dynamic effects of antibiotic treatments on both the innate and adaptive immune systems that can create opportunities

for pathogens to infect and increase disease severity that would probably lead to higher cost of treatment [3, 4].

The oral micro flora comprised of a diverse group of microorganisms with either saccharolytic or asaccharolytic metabolic lifestyles that co-exist at various niches within the oral environment [5, 6]. Surfaces of the hard tissues are colonized and inhabited predominantly by the streptococci that are equipped with external appendages that enable attachment to the tooth surface via various biochemical interactions. *Candida* spp. is both a normal member of the oral microbiota and an opportunistic fungal pathogen. It remains in the oral cavity in a nonpathogenic state for as long as the carriage percentage is kept low at 1-2% [7]. However, upon disruption of the indigenous bacterial microbiota or the host immune system, *Candida* spp. can disseminate and cause superficial infections of moist mucosal surfaces, such as those of the vagina and oral cavity [8, 9]. An important trait for the final pathogenicity of *Candida* spp. have been closely associated with the ability of these fungi to undergo phenotypic switching and produces hyphae under unfavorable condition that enable it to better adapt to the change in its growth environment [10]. In the mouth, oral thrush represents a common indication of an opportunistic infection by this fungi [11].

This study was thus carried out to assess the overall change in the distribution of microorganisms in the oral cavity before, during and after taking a 5-day metronidazole therapy. The site for specimen collection was the dorsum of the tongue, with specific focus on the distribution candidal, streptococci and other bacterial inhabitants.

## Materials and Methods

### Sample selection

Ten patients receiving antibiotic treatment at the Oral Surgery Clinic, Faculty of Dentistry, due to minor oral surgery and implant placement were selected for the study. All patients were on a five day, twice daily, prescription of 500 mg metronidazole. Patients included males and females between the ages of 20 to 50 years. Ethical approval for specimen collection was obtained from the Medical Ethics Committee, Faculty of Dentistry, University of Malaya [DF OB1503/0064(L)].

### Specimen collection

Tongue-scrape specimens from 10 patients were collected from a restricted (1x1) cm area at the centre of the tongue anterior to the sulcus terminalis before, during and a day after the completion of a 5-day metronidazole therapy. With minimal force application, a sterile excavator was used to scrape the tongue dorsum in the area specified. The excavator was then immersed in sterile Bijou bottle containing 10 ml of RTF (reduced transport fluid) and lightly shaken to dislodge the specimen from the tip and disperse the microbes. Each bottle was aseptically capped and transported to the laboratory for further processing. Specimen collection had followed the roster in Table 1.

**Table 1:** Specimen collection roster

Visits	Period on metronidazole therapy
1 <sup>st</sup> visit	<i>On the day of surgery</i>
	Tongue scrape samples were collected in the morning prior to surgery. These samples represented the baseline microbial population under the normal condition, in the absence of antibiotic.

2 <sup>nd</sup> visit	<i>Second day of antibiotic therapy</i>
	Tongue scrape samples were collected in the morning. These samples represented the microbial population during the course of an antibiotic therapy.
3 <sup>rd</sup> visit	<i>One day after completion of antibiotic therapy</i>
	Tongue scrape samples were collected on the morning of the 6th day after the surgery. These samples represented the post-antibiotic therapy samples.

### Specimen preparations

Each Bijou bottle was sonicated for 60s to disperse aggregated microbes in the specimens. The suspensions were serially diluted to 10<sup>-2</sup> using RTF as diluent.

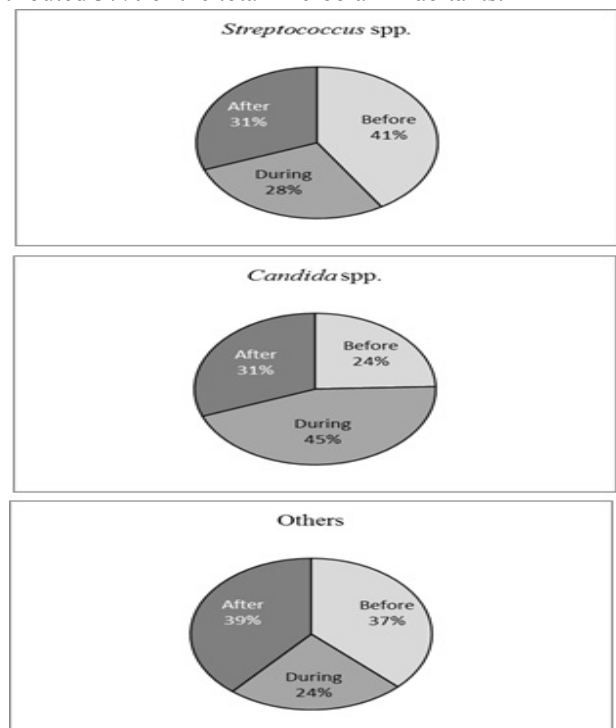
### Determination of total microbial, streptococci and candidal populations

A volume of 100 µL of the diluted samples were aseptically pipette out and inoculated onto three selective growth media plates; (i) Mitis-Salivarius agar (MSA) (Oxide, UK), (ii) Sabouraud agar (SA) (Oxide, UK) and (iii) Brain-Heart Infusion agar (BHIA) (Oxide, UK) plates for the determination of streptococci, candida and total microbial counts, respectively. Sterile cotton swabs were used for spreading of specimens on the agar plates. All plates were incubated for 18-24 h at 37°C to allow for colonies growth. Following incubation the plates were removed and the colony forming units (cfu) of each plate enumerated. Based on the cfu, the population of total microbes, streptococci and *candida* were determined. Determinations were done in triplicates for standardization purposes.

## Results

### Distribution of microbes on tongue dorsum prior to metronidazole therapy

Streptococci was identified the predominant bacteria of the tongue dorsum contributing to 41% of the total microbial inhabitants while, candida contributed the least at 24%. Other microbes contributed 37% of the total microbial inhabitants.



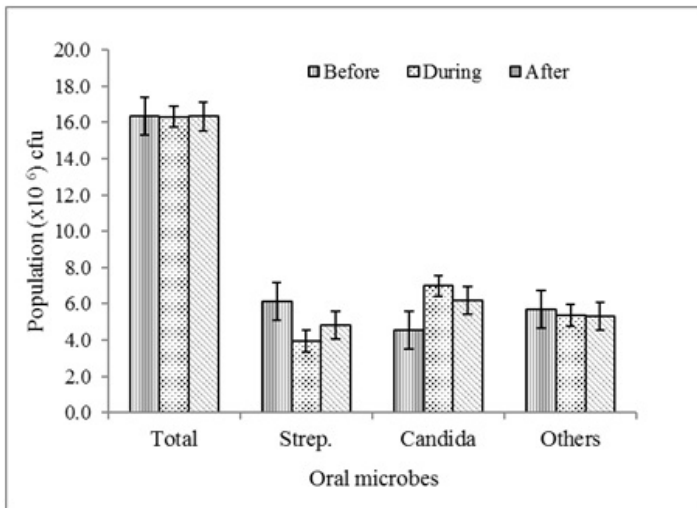
**Figure 1:** Distribution of streptococci, candida and other bacteria on tongue dorsum before, during and after a 5-day metronidazole therapy.

**Fluctuation in distribution of oral microbes in response to metronidazole therapy**

Metronidazole therapy causes shifts in the distribution of streptococci, candida and other microbial inhabitants. A significant 1.9-fold increase in the population of candida was observed upon the received of the antibiotic, accompanied by a concurrent significant reduction in the presence of the streptococci and other microbes by 1.5-foldeach ( $p < 0.05$ ) (Fig. 1).

A day after completion of the 5-day therapy, a shift in microbial distribution was again observed. The streptococci and candida attained similar level of dominancy, each contributed 31% of the total inhabitants (Fig. 2). An increased population of the other microbes was determined, contributing to 37% of the total oral microbial population.

In general, metronidazole had caused fluctuations in the distribution of oral streptococci and candida of the tongue dorsum during and after the received of the 5-day therapy. Shifts in the distribution pattern of the main streptococcal and candidal components had affected the percentage presence of the other oral microbes (Fig. 2). No significant change to the total microbial population was observed in the tongue scrape specimens before, during and after the 5-days period of antibiotic treatment (Fig. 2).



**Figure 2:** Effect of a 5-day metronidazole therapy on the total microbial inhabitants, streptococcal, candidal and other microbial components of the tongue dorsum.

**Discussion**

Oral health is dependent on the maintenance of stable microbial communities. A disturbance in this balance may result in the more pathogenic species outgrowing the commensals [12]. In other words, effects on the resident microbial ecosystem may also adversely affect patient health.

The effect of antibiotic therapy on oral microorganisms may be reflected in the changes of its microbial population either of the saliva, dental plaque or the tongue. In this study, the effect of metronidazole on the oral microbes was specifically measured

by changes in the streptococcal and candidal population on the tongue dorsum. To avoid the many factors in oral cavity that can influence the microbial composition of samples, collection site was standardised to a restricted area of (1 x 1) cm at the centre of the tongue dorsum.

The stability and homeostasis that exist between the microorganisms and host components in the oral cavity is governed by both endogenous and exogenous factors. The growth of its microbial components is especially affected by fluctuations in the pH, salivary flow, redox potential and also the availability of nutrient to maintain viability and support microbial propagation [7]. Antibiotic induces great ecological disturbance to microbial balance in the oral ecosystem. *Candida albicans* for example has been identified as an opportunistic organism and well documented as the most common cause of candidiasis and most successful yeast to colonise surfaces of the oral mucosa. This places *C. albicans* in a position that would take advantage of the immune-suppressed condition of a host. However, the isolation of candida in the oral cavity especially when in low counts does not always imply the presence of disease as the mouth has a candidal carriage rate from 2% to 71% in asymptomatic adults [13]. In medically compromised patients and those on broad spectrum antibacterial agents, the percentage has been reported to reach a 100% [7]. Other oral microbial residence is also disturbed during the course of an antibiotic treatment which may be reflected in fluctuation of their presence either in the saliva, dental plaque or tongue. As reported by Willing et al, antibiotic treatment is typically followed by a decrease in the diversity of the microbiota, and although most of the microbiota return to pretreatment levels, some members do not and are lost from the community indefinitely [14]. In this study, the effect of metronidazole on the oral microbes was specifically measured by the fluctuation in the streptococcal and candidal population on the tongue dorsum. To avoid the many factors in the oral cavity which can influence the microbial composition of the sample, collection site was standardized to a restricted area of (1 x 1) cm at the centre of the tongue.

The anti microbial actions of an antibiotic either bactericidal or bacteriostatic may kill or suppress the growth of resident bacteria, thus leaving a vacant that can be taken up by other opportunistic microbes such as the yeasts mentioned above. This was confirmed by the results obtained in this study (Fig 1). The population of *Candida* spp. which almost equals the level of streptococci before the start of therapy showed an increased with the administration of metronidazole. The main reason for this is that yeast or candida is not a true bacterium and therefore, their growth is not affected by the activity of an antibiotic like metronidazole. Thus, acting opportunistically, *Candida* spp. flourishes to occupy the vacant space. This was shown by the significant increase in the count of *Candida* by 1.9-fold in specimens collected on the second day of therapy (Fig 1). It was explained that suppression of the immune system, specifically the macrophages by antibiotics, reduces the pro-inflammatory cascade of the host that allows the spread of the pathogenic fungal form of *Candida* spp. [15]. Without macrophages to inhibit it, the fungal candida can spread more rapidly. Phenotypic switching by the candida is one characteristic that projects its supreme role as an opportunistic pathogen [10]. In contrast to *Candida* spp., the growth of streptococci was very much affected by the antibacterial activity of metronidazole. Being a true bacterium, the antibiotic effectively inhibited the growth of

streptococci as indicated by the 1.5-fold reduction in population from before to during the 5-day therapy (Fig 1).

In any disturbed growth environment, normal microbes have to adapt to the changed condition to survive and maintain viability. This suppression of growth is easily seen by an extension of the lag period of its growth curve where microbial growth becomes minimal [16, 17]. Hence, explains the slow recovery of dominance by the streptococci and the high population of candida observed even after a day the therapy ended (Fig 1). Longer period is thus, needed by the streptococci and candida to return to the initial unsuppressed growth state. Considering the status of these microorganisms as normal residents that have protective immune roles in the oral cavity, their reduced presence during a course of antibiotic therapy may increase the risk to opportunistic infection, especially in immunosuppressed patients.

### Conclusion

The course of a 5-day metronidazole treatment affected microbial balance of the oral residents. A significant rise in opportunistic species occurred, taking advantage of the disturbed bacterial balance. This study described the changes in the oral microbiota as a result of antibiotic treatment that will consequently, change the host immune homeostasis in the oral cavity. A better understanding of these changes is imperative so that side effects of antibiotic can be mitigated.

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